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Proceedings of the

Vesicant Workshop

February 1987

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Chemical warfare injuries, chemical warfare casualties, military medicine, vesicants, mustard agents, lewisite, decontamination

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The Vesicant Workshop was held at The Johns Hopkins University Applied Physics Laboratory on 3-5 February 1987. The objective of the workshop was to develop a coherent strategy to focus and prioritize research in medical countermeasures to vesicant warfare agents based on clinical data and user needs as defined by the combat developers. This document constitutes the proceedings of that workshop.

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PROCEEDINGS OF THE

VESICANT WORKSHOP,

FEBRUARY 1987

Held at the

Johns Hopkins Applied Physics Laboratory

Columbia, Maryland

Sponsored by

U.S. Army Medical Research Institute
of Chemical Defense
Aberdeen Proving Ground, Maryland
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EDITOR'S PREFACE

Because of the use of sulfur mustard by Iraq in the Gulf War and the subsequent recognition of the availability and effectiveness of vesicant agents on the battlefield, the U.S. Army Medical Research and Development Command has redirected a major part of its emphasis toward developing medical countermeasures to these compounds. The Vesicant Workshop, held on 3-5 February 1987, was an effort to lay the foundation for a comprehensive and coherent strategy to address this important military problem. During the Workshop, existing clinical and research data were reviewed, triservice requirements were identified, and a front-end analysis was conducted.

The Proceedings of the Workshop are divided into six sections: keynote address and working definitions, the vesicant injury and its management, operating in a chemical environment, deficiencies in vesicant defense, current medical research, and new directions.

A number of programmatic changes have occurred between the last day of the Workshop and the publication of these Proceedings. First, the concept of topical protectants has superceded that of topical barriers. (Topical protectants are nonclothing, nonhardware products applied to the skin or other body surface that reduce contact with a noxious agent by sorption, chemical inactivation, presentation of a barrier, or a combination of these functions. By definition, a topical protectant is applied before exposure to the noxious agent.) Second, the concept of an antidote has been added as a category of test compounds against vesicant agent effects. (An antidote is a remedy for counteracting a poison or other noxious agent. By definition, an antidote is administered after exposure to the poison.) Third, we have become aware of need for caution in fluid replacement to mustard casualties. The need to replace fluids lost from the vascular compartment because of cutaneous lesions must be offset by the possibility of pulmonary edema resulting from vesicant inhalation injury. Finally, the difficulties in obtaining comprehensive clinical data from Gulf War mustard casualties have become widely recognized.

PHILIP CHAN
COL, MC
30 November 1987

VESICANT WORKSHOP

February 1987

Applied Physics Laboratory, Johns Hopkins University
Columbia, Maryland

AGENDA

Tuesday, 3 February 1987

WELCOMING REMARKS

0800	A.	Commander's welcome	COL Joseph C. Denniston, VC USAMRDC
	B.	Keynote address	BG Richard T. Travis, MC USAMRDC
	C.	Administrative announcements	LTC Philip Chan, MC USAMRICD
	D.	Introductions	
	E.	Definitions	LTC Philip Chan, MC USAMRICD

SESSION I: THE VESICANT INJURY AND ITS MANAGEMENT

Moderator: Dr. John S. Urbanetti

0900	A.	Injury to skin, immune system, and internal organs	CPT Kenneth G. Phillips, MC USAMRICD
0930	B.	Injury to the pulmonary system	John S. Urbanetti, M.D. Southeastern Pulmonary Association
0945	BREAK		
1000	C.	Injury to the eye	LTC William R. Rimm, MC WRAIR, and Maj Charles F. Bahn, USAF, MC USUHS

1015	D.	Recent experiences in mustard casualty management	COL David L. Bunner, MC USAMRIID
1030	E.	Treatment of the cutaneous injury	COL Basil A. Pruitt, MC U.S. Army Institute of Surgical Research
1050	F.	Discussion	
1130	LUNCH		

SESSION II: OPERATING IN THE CHEMICAL ENVIRONMENT

Moderator: MAJ Daniel L. Rickett

1230	A.	Threat briefing (classified)*	Mr. William Feeney Foreign Science and Technology Center
1245	B.	Military operational doctrine	MAJ Merrill S. Blackman, CM U.S. Army Chemical School
1300	C.	Implications for Naval assets and operations	CAPT W.M. Parsons, MSC, USN Naval Medical Command
1310	D.	Implications for Air Force assets and operations	Lt Col Gary R. McNutt, USAF, BSC HQ USAF
1320	E.	Medical considerations and implications	Robert H. Mosebar, M.D. Academy of Health Sciences
1350	BREAK		
1405	F.	Discussion	

*Classified presentations do not appear in this Proceedings Volume.

Wednesday, 4 February 1987

0800 ADMINISTRATIVE ANNOUNCEMENTS

SESSION III: DEFICIENCIES IN THE DEFENSE AGAINST VESICANTS

Moderator: Dr. Robert H. Mosebar

0815	A.	The TRADOC Combat Developer's perspective	MAJ Merrill S. Blackman, CM
0830	B.	Deficiencies in the medical response	Robert H. Mosebar, M.D.
0845	C.	Joint Services Agreement (JSA) requirements and priorities (classified)*	MAJ Daniel L. Rickett, MS USAMRICD
0900	D.	Discussion	
0945	BREAK		

SESSION IV: CURRENT MEDICAL RESEARCH

Moderator: LTC Michael J. Reardon

1000	A.	Mechanism of action of sulfur mustard	Bruno Papirmeister, Sc.D. Science Applications International Corp.
1020	B.	Current skin decontamination development program	LTC Donald G. Harrington, VC USAMMDA
1040	C.	Animal models for vesicant injury	MAJ Dale R. Westrom, MC LAIR
1100	D.	Army's current research program in medical countermeasures to vesicants	LTC Michael J. Reardon, VC USAMRICD
1115	LUNCH		
1215	E.	International vesicant research	Bruno Papirmeister, Sc.D.
1230	F.	Discussion	

*Classified presentations do not appear in this Proceedings Volume.

SESSION V: NEW DIRECTIONS (discussion groups)

Moderators: LTC Gerald L. Wannarka, MS, USAMMDA
MAJ Daniel L. Rickett

Facilitator: LTC Philip Chan

1330 A. Programmatic research deficiencies

Thursday, 5 February 1987

0800 ADMINISTRATIVE ANNOUNCEMENTS

SESSION V: NEW DIRECTIONS (continued)

0815 B. Vesicant research program goals and objectives

1200 LUNCH

1300 C. Discussion

1400 D. Closing remarks — COL Joseph C. Denniston, VC

KEYNOTE ADDRESS

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In this age of nuclear-tipped cruise missiles, Trident submarines, other devastating weapons of mass destruction, and nerve agents that can kill instantly, it is the vesicants that present the greatest threat to unprotected and partially protected soldiers in low and moderate intensity combat. History tells us that mustard on the battlefield dramatically undermines the ability of medical facilities to survive and to function at maximum efficiency while preserving the fighting strength and facilitating the return to duty.

Mustard's threat is critical, partly because it incapacitates so many more combatants than it kills and partly because it is so easily prepared from commercially available chemicals. Recently, a Belgian company was alleged to have exported 500 tons of thiodiglycol to Iraq in 1983. This chemical, when combined with hydrochloric acid, produces mustard in excellent yield. Clearly, the synthesis of sulfur mustard is within the capability of any Third World country.

In November of 1983, Iran reported to the United Nations that its soldiers had been attacked with chemical agents (mustard and tabun) by Iraqi forces. The Secretary General of the United Nations unilaterally invited ten nations to send a team to Iran to investigate their claim. Spain, Australia, Sweden, and Switzerland accepted, and their representatives proceeded to Iran. The results of their visit are contained in United Nations Security Document S16433, entitled, "Report of the Specialists Appointed by the Secretary General to Investigate Allegations by the Islamic Republic of Iran Concerning the Use of Chemical Warfare," dated 26 March 1984.

Our purpose during these 3 days is to develop a coherent, hard-hitting, no-nonsense investment strategy to focus and to prioritize research in medical countermeasures to vesicant injury. You should pay particular attention to user needs as defined by the combat developers, joint service agreements, and other DoD thrusts.

In addition, you should recognize that there are severe fiscal restraints on this Command which mandate that all programs be tailored for maximum efficiency and productivity. This will involve internal and external realignment, and the reprogramming of resources brought to bear on these problems. We must be flexible, but I want to proceed in an orderly, coherent, rational fashion. We have already begun this realignment and reprogramming process.

As you consider the threat, please be sensitive to the vulnerability of the Health Care Delivery System in the AirLand Battle 2000 Concept. As our maneuver battalions attempt to control the FLOT (Forward Line of Own Troops), particularly along the NATO defensive positions, innovative deployment of limited medical resources will be required. Please be advised that there are very few medical personnel per battalion, zero guarantees of 100% air superiority, and reduced availability of MEDEVAC helicopters. Personnel must be able to fight in a chemical environment and survive vesicant injuries.

Current decontamination concepts for non-fixed health care facilities leave much to be desired, but in fast-moving combat, that's reality. The chemical agent monitor (CAM) is still in development, and the ability to detect agent on patients, collective protective shelters, or softsided battalion aid stations is marginal at best. Currently fielded is the M258A1 decontamination kit, and a joint development (Cml Corps/MRDC) replacement item is in the pipeline.

Research needs to be focused. This does not mean that this Command is not interested in intellectual products that enhance the tech base--that's our seed corn and we will not abandon it--but rather that we need to have concrete plans for a short-term fix and crystal-clear mid- and long-term research directions.

Finally, MG Russell and I guarantee the support of this Command in your endeavors. You have assembled here a critical mass of outstanding scientists. I charge you to accept the challenge to be perceptive, creative, innovative, and realistic in your efforts here, while keeping in mind that the medical community's ability to manage vesicant casualties in the future will be based upon the results of your work now.

WORKING DEFINITIONS OF CATEGORIES OF PROTECTION AND TEST COMPOUNDS

A. Topical barrier: Nonclothing, nonhardware product applied to the skin or other body surface that reduces contact with a noxious agent.

B. Decontamination: Physical or chemical removal of a noxious agent. By definition, decontamination follows exposure to the noxious agent.

C. Detoxification: Irreversible inactivation of a noxious agent.

D. Pretreatment: Pharmaceutical or other medical product that makes a person less susceptible to the effects of a noxious agent. The expected pretreatment period is short (hours to days, maximum of 3 weeks), and a pretreatment is used when exposure to the harmful agent is imminent.

E. Prophylactic: Pharmaceutical or other medical product that makes a person less susceptible to the effects of a noxious agent. The period of prophylaxis is long-term (weeks to years).

F. Treatment: Pharmaceutical or other medical product that has a beneficial effect on casualties.

G. Medical management: Medical support of the whole patient.

H. Definitive care: Comprehensive medical care to facilitate return to duty and recovery of function.

Session I

The Vesicant Injury and Its Management

TITLE: Injury to the Skin, Immune System, and Internal Organs

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When the troops of the American Expeditionary Force (AEF) left for France under the command of General "Blackjack" Pershing, they had already trained in their protective masks and had been advised of the risks they were to face. Yet, on arrival, they found their protective equipment to be ineffective. They were immediately issued French masks, and it was with these masks that they faced their first gas attack.

Gas was the number one cause of nonfatal casualties and the number four cause of death in the AEF (Prentiss, p. 669). Mustard probably accounted for 60-80% of the Allied Forces gas casualties.

The eyes proved to be the organ most sensitive to mustard, followed by the respiratory tract and the skin. The average duration of hospitalization for mustard in the AEF was 60 days. Postmortem findings of those dying within 48 hours of exposure were most remarkable in the pulmonary system.

Lewisite differs from mustard in that pain occurs on contact and the blister fluid contains arsenic. Damage is done with mustard hours before the first sign or symptom, and the fluid in the blister contains no active mustard. Although healing is slow with both agents, mustard blisters are reported to heal more slowly.

Phosgene oxime causes almost intolerable pain and local tissue destruction immediately on contact with skin and mucous membranes.

INJURY TO THE SKIN, IMMUNE SYSTEM, AND INTERNAL ORGANS
Presented by CPT Kenneth G. Phillips, MC

CPT Phillips discussed the characteristics and similarities of three vesicant agents: mustard, lewisite, and phosgene oxime. Important points included:

- Of the types of battle gas used in World War I (vesicants, lung injurants, choking and pain-producing agents, and lacrimators), vesicants produced the most casualties per pound of gas (Prentiss, p. 662).
- More than 450,000 Russian soldiers were chemical casualties in World War I.

Mustard (H):

- Most lesions in the military arena are caused by exposure to vapor.
- An incapacitating conjunctivitis can occur at the odor threshold.
- The odor threshold can be raised by fatigue; therefore, it is conceivable that troops could be exposed to incapacitating levels without knowing it.
- Vesication dose = 10 ug/cm^2.
- Inhalation LD_{50} = 1500 $mg/min/m^3$.
- Because of slow excretion rate, repeated low doses have a cumulative effect.
- Effects on the eye can range from mild conjunctivitis to corneal necrosis and opacification.
- Inhalation produces damage primarily to tracheal and bronchial mucosa.

- Ingestion of mustard produces necrosis and desquamation of gastrointestinal mucosa.
- Relative skin sensitivity is directly related to relative thickness.
- The most sensitive skin is in areas that are warm and moist (Medical Aspects of Gas Warfare, p. 69).
- Epidermal healing results from epithelial regrowth from the margin of a lesion and from skin appendages.
- Fluid from mustard-produced vesicles is not toxic and does not produce vesication.
- Vesicant injury healing is delayed compared to that of thermal burns.
- There are often problems with local and systemic infection.

Lewisite (L):

- Lewisite mimics mustard effects in some ways.
- Lewisite causes almost immediate pain on contact with the skin and with mucous membranes of the eyes, nose, and throat.
- Fluid from lewisite-produced vesicles is toxic because it contains arsenic, but it does not cause vesication.
- The odor threshold is usually near the threshold for irritation of the mucous membranes; and at a concentration high enough to produce skin burning, the odor of lewisite can be detected by most individuals.
- Cutaneous exposure may produce pulmonary edema that develops 2-18 hours after exposure.
- Patients contaminated with lewisite have the additional problem of systemic arsenic absorption.
- British anti-lewisite (BAL) works well against this compound and can be administered topically as an ointment or parenterally by intramuscular injection.
- Skin ulcers may be bright red with multiple hemorrhages at the base. Secondary infections of skin lesions are rare.

Phosgene oxime (CX):

- Phosgene oxime produces lesions much like a nettle sting.
- Phosgene oxime causes immediate pain after contact with the skin and mucous membranes. The skin blanches in 5-20 seconds, and a wheal develops in 5-30 minutes. Dark eschars may be seen in 5-7 days. If cutaneous ulcers develop, they tend to be deep and pitted. Healing is very slow and sometimes remains incomplete 4-6 months after exposure.
- Phosgene oxime has rapidly incapacitating effects.
- Phosgene oxime penetrates garments better than mustard.
- Enough phosgene oxime can be absorbed through the skin to produce systemic poisoning and death.
- Either dermal contact or inhalation can produce pulmonary edema within 2-24 hours.
- The LD_{50} is estimated to be 30 mg/kg.

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TITLE: Vesicant Injury to the Respiratory System

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Although vesicant use during World War I resulted in numerous military and civilian casualties whose long-term morbidity was largely due to the topical effects of vesicants on the eyes and skin, the majority of the short-term morbidity/mortality of vesicant exposure was due to the respiratory effects of these agents. Inhalation of vesicant vapors produced respiratory tract effects, with a time course and degree of severity roughly equivalent to that seen with ocular exposures to the vapor. A relatively low death rate (approximately 1%) was felt to be attributable to the relatively high boiling point of mustard, resulting in generally low vapor concentrations in areas of exposure. As a consequence, much of the subsequent research regarding vesicants was directed to the development of devices to increase the vapor concentration (and hence respiratory exposure) in combat environments.

Subsequent to World War II, the reported use of vesicants has been in relatively warm environments--a factor believed to have increased the frequency and severity of respiratory exposure. Therefore, further study of the effects of these agents on the respiratory system has become an important consideration. Most of the clinical and research data regarding vesicant exposures were collected during the period from World War I to the end of World War II. Subsequent data collection has been severely limited by political constraints.

Acute respiratory effects of vesicants:

1. Upper airway: sneezing, lacrimation, rhinorrhea, epistaxis, sore throat, and hoarseness

2. Lower airway: hacking cough, tachypnea, pseudomembrane formation, and pulmonary edema

Chronic respiratory effects of vesicants:

1. Upper airway: hoarseness, loss of taste and smell, recurrent epistaxis, laryngeal carcinoma, and cicatricial lesions of the trachea

2. Lower airway: chronic nonproductive cough, bronchitis, and bronchial carcinoma

Available therapeutic interventions are limited to symptomatic treatment only. There are no available therapies for decontamination, detoxification, pretreatment, or prophylaxis of the respiratory effects of vesicants.

RESPIRATORY EFFECTS OF VESICANT INHALATION
Presented by John S. Urbanetti, M.D.

Dr. Urbanetti's presentation focused on the effects of sulfur mustard on the lungs. Using information obtained from physicians who had treated Iranian casualties exposed to mustard, he established the role of pulmonary effects in the overall picture of mustard exposure. He also compared the respiratory effects of mustard with the effects of lewisite and phosgene oxime.

Effects of vesicant inhalation include the following:

General:

- Deaths by inhalation of vesicants are few.
- Respiratory effects are a major contributor to the clinical illness seen after vesicant exposure.

Mustard:

- Mustard is an oily liquid (15°-215°C) with an odor threshold of 0.6 mg/m^3 and a lethal threshold of 100-200 mg/m^3 for a 10-minute vapor exposure.
- The odor threshold is not protective with respect to mustard (damage can occur at levels below the odor threshold).
- Mustard battlefield deaths comprise only 1-2% of the exposed population and are attributable to acute respiratory effects.
- Vapor lingers for a substantial period of time.
- Warmer environments are more conducive to maintaining high vapor concentrations, which are believed to lead to higher rates of respiratory injury.
- Conceivably, soldiers could accumulate a dose toxic to the respiratory tract after 5-6 days of low-level exposure to vapors emitted by simple ground contamination.

- The earliest acute respiratory effects of mustard exposure appear within 2-4 hours. The principal complaint at this time is chest tightness and oppression; sneezing, lacrimation, rhinorrhea, epistaxis, hoarseness, and hacking cough also develop. Sinus pain and severe epistaxis develop at 4-16 hours; severe cough, aphonia, and tachypnea, at 16-48 hours; severe dyspnea and pulmonary edema appear at 24-48 hours after exposure; and bronchopneumonia develops at 48-72 hours.

- The evolution and timing of respiratory and ocular effects coincide.

- Possible chronic effects include: hoarseness with laryngeal irritation, chronic bronchitis, fibrosis and scarring of denuded respiratory epithelium, and loss of taste and smell.

- There is an increased incidence of laryngeal and bronchial cancer with long-term chronic exposure.

- In 95% of 233 Iranian patients, a primary complaint concerned the respiratory tract. (Editor's comment: Presumably, their chemical protection was suboptimal.)

- Ocular effects were found in 92% of these patients and dermal effects in 83%.

- Acute tracheobronchitis, found in 83% of patients, was the major respiratory complaint in the above population. Asthmatic bronchitis was present in 2%, pneumonia in 1.5%, and adult respiratory distress syndrome in 1%.

- Chest X-ray was determined not to be a reliable tool for assessing the degree of mustard exposure because acute changes are central in the tracheobronchial epithelium, not peripheral in the lung parechyma.

- White blood count may be misleading; i.e., an exposed patient may have a normal white cell level initially but be markedly leukopenic after 5-10 days. Death may supervene as a result of granulocyte depletion and pulmonary and systemic bacterial infections. The decrease in lymphocytes may be present but is clinically less important--patients rarely die from fungal or tubercular infections because the lymphopenia does not persist long enough to be clinically relevant.

- Data on three mustard fatalities are shown in Table 1.
- Seventy-eight percent of 236 Iranian patients had persistent respiratory effects 2 years after exposure, as shown in Table 2. (There is some question about the accuracy of the numbers reported, but the symptoms reported are reliable.)
- Mustard exposure inflames and irritates bronchial tissue.
- Mild exposure causes bronchial inflammation, moderate exposure produces pseudomembranes, and severe exposure results in necrosis.
- Secondary infections are common after inhalation of mustard.
- Diphtheritic pseudomembranes may peel off, dislodge, and produce respiratory obstruction.
- No specific therapy is currently available for mustard inhalation.
- Symptomatic treatment includes cough suppressants (codeine) and bronchodilators.
- No pretreatment or prophylaxis is available for mustard inhalation.
- Antibiotic treatment is important to treat bonafide, established respiratory bacterial infections but does not prevent then.

Lewisite:

- Lewisite inhalation produces the same respiratory effects as mustard inhalation.
- Respiratory effects are less likely in combat with lewisite than with mustard because of the compound's volatility (shortening its persistence) and because of the immediate onset of symptoms after exposure (warning soldiers to mask).
- Topical or inhalational exposure to lewisite can produce pulmonary edema (this may be an arsenic effect).

- Pulmonary edema is treated as any other noncardiac pulmonary edema.
- Canine studies suggest that parenteral BAL within 90 minutes of exposure may reduce respiratory toxicity from cutaneous and inhalation exposures.

Phosgene oxime:

- Very little information is available on the pulmonary effects of phosgene oxime.
- Pulmonary edema can result from inhalation and from cutaneous exposure.

NOTE: A paper on this topic by Dr. Urbanetti is in press (Urbanetti 1987, listed in the references).

Table 1. Findings in Three Mustard-Related Fatalities; Iran, 1986
(Adapted from Balili-Mood, Farhoodi, and Panjvahi 1986)

	Patient		
	No. 1	No. 2	No. 3
Age (years)	27	18	17
Burns (%)	>85	35	15
Tracheobroncheal injury	3+	4+	3+
Bronchopneumonia	3+	4+	3+
White blood count			
Initial	16,800	27,000	18,900
Terminal	0	450	1,200
Time to death (days)	8	9	11
Cause of death	Septic shock	Pulmonary embolus	Adult respiratory distress syndrome

Table 2. Delayed Mustard Effects in 236 Iranian Patients 2 Years after Exposure
(Adapted from Balili-Mood 1986)

Effect		Percent occurrence
Respiratory		78
	Chronic bronchitis	38
	Asthma	29
	Rhinopharyngitis	12
	Tracheobronchitis	11
	Laryngitis	7
	Recurrent pneumonia	5
	Bronchiectasis	2
CNS		45
Skin		41
Eyes		36

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TITLE: Vesicant Injury to the Eye

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In general, chemical injuries to the eye produce incapacitation because of pain from corneal epithelial defects and loss of vision from profuse tearing and the disruption of the corneal epithelial surface. The only effective preventive measure is to limit exposure by covering the eye. The only effective initial treatment is the immediate removal of the chemical from the eye by copious irrigation with any nontoxic fluid available and the debridement of solid particles.

The length of incapacitation and final visual outcome depend on the nature of the chemical itself and the length of time the eye is exposed to the chemical. For example, acids are neutralized quickly at the ocular surface while bases are not. Bases, therefore, continue to cause damage until they are "diluted out" by ocular tissues and generally produce more serious injuries than do acids. In a case of brief exposure to a weak acid, the eye pain is severe and vision is completely blurred for 24 to 48 hours, until the corneal epithelial surface is repaired. At the other extreme, a severe lye injury causes severe eye pain, and the eye progressively "melts" and vision is irreversibly lost over several days. In intermediate cases of lye exposure, the eye may not be irreversibly damaged but remains painful for weeks, and vision remains poor because of progressive vascularization and scarring of the cornea. In some of these cases, it may be possible to restore vision about a year later by corneal transplantation, although the prognosis is very guarded because of the high incidence of late secondary ocular sequelae such as glaucoma.

Ocular injuries from vesicant warfare agents pose some special problems. While vesicants penetrate the ocular tissues within 2 to 5 minutes, the onset of clinical manifestations ranges from immediate to delayed, with latent periods from 2 to 48 hours after exposure, depending on the agent and degree of exposure. Ocular findings range from a mild conjunctivitis, to corneal opacification and scarring, to the rare loss of the eye. Treatment is supportive, with cycloplegics, antibiotics, and topical steroids having roles in appropriate cases. Except with respect to lewisite, no effective *in vivo* detoxicant is available for vesicant injuries to the eye. To be effective against lewisite, BAL must be applied within 2 to 5 minutes after exposure.

VESICANT INJURY TO THE EYE

Presented by LTC William R. Rimm, MC
and Maj Charles F. Bahn, USAF, MC

LTC Rimm and MAJ Bahn discussed the effects of vesicants on the eye. This included an anatomical overview and comments on most clinicians' lack of training in the management of vesicant-induced eye injuries.

MAJ Bahn:

- Few military and civilian ophthalmologists are aware of the ocular effects of vesicants (particularly the delayed effects of mustard).

Physiological considerations/principles include the following:

- The corneal epithelium is very richly innervated.
- The anterior corneal surface is the most important refractive surface of the eye.
- The cornea is not passively transparent, like glass, but its lucidity depends on its viability.
- The endothelium continually pumps fluid from the corneal stroma back into the anterior chamber. If the endothelium is damaged, the cornea retains water and rapidly becomes opaque, resulting in loss of vision.
- Most ophthalmologists are trained to treat acid and base injuries to the eye, in which rapid irrigation ameliorates the injury.
- Acid injuries are generally neutralized at the surface.
- Alkalis damage by deep penetration and are neutralized by dilution in ocular tissue.
- Injuries from bases tend to be more significant than injuries from acids.
- Irrigation, which is very important in the treatment of acid and base injuries, may not be important in the treatment of vesicant-induced eye injuries.

LTC Rimm:

- Lewisite

  a. The corneal surface is free of unbound toxin within 2-4 minutes.

  b. Toxin is found within the corneal stroma and the anterior chamber within 2 minutes.

  c. The anterior chamber is free of unbound toxin within 30 minutes.

  d. Some toxin remains within the corneal stroma for 1-26 hours.

- Vesicants penetrate the eye quickly, have their effect, and quickly exit.

- The eye is free of unbound mustard within 15 minutes.

- Mustard's mechanism of action: Intracellular alkylation has profound effects on DNA replication and causes irreversible histologic changes within 30 minutes.

- Hydrochloric acid liberated during reactions of both mustard and lewisite lowers pH and also has its own minor effect.

- Speculation: One explanation for the delayed onset of mustard eye symptoms is that the epithelium remains intact over a fluid layer for several hours after exposure to mustard. Conceivably, the patient becomes symptomatic only after the epithelium sloughs and corneal nerves are exposed.

- By the time the patient experiences symptoms from mustard, the irreversible damage has already occurred. There is little that can be done at this point except symptomatic treatment.

- In contrast, lewisite and phosgene oxime both have rapid onset of symptoms.

- There is nothing specifically effective for the treatment of mustard injuries to the eyes.

- Mustard eye casualties:

  a. 75-90% of all mustard casualties will have ocular involvement, with the delayed onset of symptoms peaking 6-12 hours after exposure.

  b. 90% of eye casualties will have minimal corneal involvement and no permanent ophthalmologic sequelae.

  c. 90% of eye casualties will remain incapacitated for 10-14 days.

- Symptomatic impediments to the satisfactory performance of military duties during the 10-14 days of incapacitation include: gritty sensation in eyes, conjunctivitis, exudate, blurred vision, blepharospasm, and photophobia.

- Significant corneal involvement (which may progress to corneal edema and, rarely, loss of the eye) will occur in approximately 10% of mustard eye casualties.

- Those patients with significant corneal involvement are incapacitated with injuries that are considered moderate to severe and may require hospitalization for up to 4 months.

- Severe vesicant effects to the eye may involve scarring between the iris and the lens, restricting pupillary movement. Also, scarring that blocks the movement of aqueous humor out of the anterior chamber predisposes those persons to glaucoma (rare).

  Recurrent erosions/ulcerations are possible 20-30 years after the injury. These are similar to recurrent erosions seen after injuries caused by contact with certain vegetables and may involve the basement membrane.

Progression of Symptoms and Clinical Findings:	Degree of Incapacitation:
1. Redness	Able to return to duty soon
↓	
2. Corneal edema (interferes significantly with vision)	Usually able to return within several days to 2 weeks
↓	
3. Inferior pannus development (vascularization of the cornea causing corneal opacity). There are varying degrees, i.e., visual axis involvement, total opacification, interstitial keratitis with possible calcification	If pannus covers visual axis, patient would lose vision of that eye
↓	
4. The eye may ulcerate; possible prolapse of uveal tissue through the cornea. The eye may be salvageable, but significant visual problems are likely	Will require prolonged hospitalization and probable medical discharge

- If a patient has moderate to severe ocular effects, he is at increased risk of developing systemic effects because of inhalation of agent.
- The latent period before the onset of symptoms with mustard exposure causes many problems of diagnosis, triage, and treatment.

Management:

- Management options are very limited; injuries need to be treated early (this is unlikely with mustard injuries because of the delayed onset of symptoms).
- Copious irrigation should be started within 2-5 minutes.

- British anti-lewisite is the only effective and specific medical detoxicant available for a vesicant, and it is only effective against lewisite: 1-25% will prevent corneal opacification; 5% is optimum; 3-10% is tolerated; >15% causes corneal edema.
- Early short-term use of topical steroids may be effective.
- Use of topical anesthetics should be avoided because of their cumulative toxic effects on epithelium.
- The available literature suggests that the respiratory symptoms may be somewhat delayed compared to the ocular symptoms. Therefore, anyone with moderate to severe ocular injury should be monitored for the development of respiratory problems.
- Eyes should be irrigated as soon as possible after exposure. Recommendation: Irrigate the eyes of everyone with the chance of recent exposure.
- Patients with mild eye injuries seldom require hospitalization but they are still likely to be restricted from full military duty for up to 2 weeks.

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Clinical and laboratory findings in Iranian fighters with chemical gas poisoning (Arch. Belges Suppl., p. 254, 1984).

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Introduction and outline

Primary reaction of mustard with the corneal epithelium

The histopathology of the ocular lesions produced by the sulfur and nitrogen mustards

Effects of mustard and nitrogen mustard on mitotic and wound healing activities of the corneal epithelium

Nuclear fragmentation produced by mustard and nitrogen mustards in the corneal epithelium

Note on karolysis of the corneal stroma cells

The adhesion of epithelium to stroma in the cornea

The effect of histamine and related substances on the cohesion of the corneal epithelium

Loosening of the corneal epithelium after exposure to mustard

Exploratory studies on corneal metabolism

The effects of mustard on some metabolic processes in the cornea

Further experiments on corneal metabolism in respect to glucose and lactic acid

The consumption of pyruvate, acetoin, acetate, and butyrate by the cornea

The utilization of ribose and other pentoses by the cornea

TITLE: Recent Experiences in Gulf War Casualty Management

AUTHOR: COL David L. Bunner, MC

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Chemical warfare has apparently been accepted in some Western countries as permissible, as measured by a willingness to supply needed agents/reagents. A large number of victims of vesicant and nerve agents have received care from countries friendly to the United States. Problems in diagnostic as well as therapeutic approaches are apparent from the results. Important information can be gained by attention to recent field and clinical experiences. A need for basic, applied, and clinical research is evident. Clinical education and experience for military physicians/researchersare also desirable. Problems of pulmonary and systemic injury, late exposure of health care personnel, and clear clinical differences from mycotoxin exposure will be discussed.

RECENT EXPERIENCES IN GULF WAR CASUALTY MANAGEMENT
Presented by COL David L. Bunner, MC

Among the Workshop participants, COL Bunner had the unique opportunity of observing the medical management of Gulf War vesicant casualties in European hospitals. In the early 1980s, it was rumored that a low molecular weight toxin was responsible for injuries that had been sustained by many Iranian casualties. COL Bunner's consultation was requested because he has extensive knowledge of the T2 mycotoxin injury in laboratory animals and humans. Upon examination of the Iranian casualties, however, COL Bunner determined that a mycotoxin was not the etiologic agent, and that mustard clearly was. The following observations and conclusions are based on COL Bunner's observations in Europe.

Observations:

- Vesicants should be recognized as likely threats: they are easy to synthesize and their component materials are readily available to all industrialized and unindustrialized nations.
- Physicians require hands-on experience for diagnosing and treating vesicant injuries and for promoting research in this area. This experience should be provided to at least a core group of military physicians.
- Good basic science data are needed to devise objective approaches for the diagnosis and treatment of vesicant injuries.
- The approaches for treating recent vesicant casualties have been empirical and highly variable.
- U.S. physicians have not had the opportunity to obtain significant clinical experience with vesicant casualties.
- Some medical facilities where vesicant casualties were treated lacked the equipment needed for good data collection.
- Obtaining a definitive diagnosis by analytical testing may take weeks to months.

- The issue of agent persistence and adequacy of decontamination must be addressed: secondary dermal injuries were observed by European medical personnel up to 7 days after the Iranian casualties arrived. (Editor's note: Secondary skin injury probably resulted from inadequate decontamination of casualties.)

- Vesicants also cause systemic injuries. Since these injuries are delayed, a reasonable time window may exist for treating them.

Conclusions:

- The recent chemical casualties treated in European hospitals are a potentially valuable resource for obtaining clinical data. Every effort should be made to obtain and record pertinent information.

- This newly obtained data should be considered when devising a new program for development of practical field approaches.

- Discrepancies and omissions in the FM 3-5 Manual should be corrected since they promote confusion and guesswork in chemical casualty management.

- The management of vesicant casualties should receive an especially high priority in the clinical and field training of military physicians because of the current use of mustard in the Gulf War and the ease with which mustard can be manufactured and deployed.

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TITLE: Treatment of Cutaneous Vesicant Injury

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Burns are common combat-incurred injuries (5-18% of all casualties in recent conflicts) that can rapidly incapacitate a soldier. The physiologic responses, intensity of medical care, and mortality are proportional to the extent of the body surface injured. In patients with conventional burns, systemic support takes priority in the immediate postinjury period, but in patients with chemical burns, particularly those caused by vesicants, treatment of the burn per se takes precedence in order to limit systemic absorption and minimize local tissue injury. All contaminated clothing must be removed and all skin exposed to the agent immediately lavaged with copious amounts of water. Vesicles should be debrided during the cleansing procedure to prevent injury to contiguous areas by serous fluid containing the vesicant. Subsequent treatment of the cutaneous injury is the same as for any burn, with emphasis on prevention of infection and early closure of the wound.

Inhalation injury can also be produced by vesicants, necessitating tracheal intubation and mechanical ventilatory support. The severity of airway damage and need for intubation can be rapidly assessed by endoscopic means, as can vesicant-induced injury of the upper gastrointestinal tract. Triage criteria are presented to guide the efficient application of available medical resources in the treatment of soldiers with cutaneous injury.

NOTE: A treatment outline by Dr. Pruitt is in Appendix A.

TREATMENT OF THE CUTANEOUS INJURY

Presented by COL Basil A. Pruitt, Jr., MC

COL Pruitt discussed both thermal and chemical burns and emphasized differences in treatment. Initial triage, acute management, and follow-up therapy were presented.

Details of the presentation included the following:

- Two reasons why the military has a particular interest in burns are that: 1) a large number occur in combat (probably one of ten injuries is a burn); and 2) since burn injuries affect all organ systems in the body, the burn patient is the universal trauma model.
- The magnitude of the physiologic response is proportional to the extent of the burn: a readily estimable severity index is possible.
- Early care of the chemical burn patient:
  1. Prevent further injury:
     a. Remove all clothing.
     b. Undertake copious lavage to decontaminate.
  2. Assure patency of the airway.
  3. Start fluids; i.e., support the circulation to maintain vital organ function.
  4. Protect the patient against pathophysiological changes such as dilation of the stomach and bladder.
  5. Excise nonviable tissues: Tops of bullae > 2 cm should be debrided under running or standing water.
- Chemical injuries differ from thermal burns in that they require attention to the local wound in the immediate post-injury period.

- The severity of cutaneous chemical injury is determined by the concentration, quantity, and duration of contact with the agent. The severity of airway and lung injury, due to inhalation of volatile or aerosolized agents, is determined by the same factors.

- The clinical consequences of chemical injury depend upon the amount of skin surface affected, the functional importance of the tissue involved (e.g., hands, feet, face, and the organs of special sense), and the severity and extent of pulmonary injury.

- If there is any agent present when the patient is first seen, it should be removed by copious water lavage after removing all clothing.

- No neutralizing agents should be used to detoxify vesicants on the skin if the heat of an exothermic chemical reaction would cause an additional thermal injury.

- Eye injuries should be irrigated, and the patient should be given supportive treatment as if the injury had just happened.

- There is some controversy about whether the fluid in a mustard-induced vesicle is toxic. COL Pruitt has seen what appeared to be vesication caused by blister fluid; therefore, he recommends that blisters be opened while they are being lavaged.

- Sulfamylon® cream (mafenide acetate) is the recommended antibiotic for the topical treatment of vesicant burns because of its broad spectrum of antibacterial activity. Silvadene® cream (silver sulfadiazine) is an acceptable alternate, but its spectrum of antibacterial activity is narrower.

- Inhalation injuries are hard to diagnose; the signs are most evident 2-3 days after exposure.

- The site of injury within the airway due to inhalation of an aerosol is dependent on particle size, pattern of breathing, and other factors.

- Follow-up care of the patients with second and third degree burns:

  1. After resuscitation, one can estimate the amount of fluid needed to replace the insensible water loss of a burn patient according to the formula:

     (25 + % burn) x total body surface (m^2) = ml/hr

  2. Because of higher than normal metabolic needs, the burn patient, particularly those with >45-50% of body surface burned, has markedly elevated caloric and protein requirements necessitating vigorous nutritional support.

- Triage for second and third degree burns in mass casualty situations:

a. If patient has relatively small amount of body surface burned (1-20%)	Delay hospital care
b. If patient has >60% of body burned	No treatment
c. If patient has 20-60% of body burned	Treat immediately

- In a combined injury, the systemic effects of vesicants may accentuate the systemic responses caused by thermal burns.

NOTE: A treatment outline by Dr. Pruitt is in Appendix A.

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DISCUSSION

Session I

Most of the discussion after the presentations in Session I was related to the use of thermal burns as a model for vesicant-induced injuries and the adequacy of doctrine in the triage and management of vesicant injuries. Vesicants can produce lethal systemic effects without producing dermal blisters; for example, inhalation of vesicant produced fatalities among Bari Harbor casualties and men who inhaled smoke from firewood impregnated with mustard. In addition, unlike thermal burns, mustard injuries rarely involve full skin thickness.

Constitutional and systemic effects begin to occur with as little as 20 cm^2 of skin exposed to mustard. Under normal circumstances, 80% of the mustard that contacts the skin will evaporate, leaving 20% to penetrate the skin. Only 2% of the total exposure quantity is fixed in the skin, leaving 18% of the total to be absorbed in the circulation, available to produce systemic effects. A lethal systemic dose of mustard is only a few milligrams per kilogram; therefore, if mustard in an open wound is absorbed, it could penetrate rapidly into the circulation in concentrations sufficient to produce serious systemic effects. Percent body involvement was felt by some workshop participants to be of little quantitative usefulness because of the difficulty in relating affected surface area to total dose accumulated. In addition to the percent of body surface exposed, the total toxic dose would depend on the concentration and duration of both cutaneous and inhalation exposures.

Liquid versus vapor exposure was discussed. It was noted that patients exposed to liquid or droplets may benefit from decontamination, but persons exposed only to vapor will not. The severity of a vesicant skin injury is a function of how much mustard is fixed per square centimeter of skin; it does not matter whether the mustard comes from liquid or vapor exposure. Systemic effects will develop as a dose-related consequence of exposure. An adequate and objective measure of the vesicant injury for triage and prognosis has not yet been developed and should be considered.

Session II

Operating in the Chemical Environment

MILITARY OPERATIONAL DOCTRINE

Presented by MAJ Merrill S. Blackman, CM
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MAJ Blackman presented an overview of national policy and military operational doctrine as it relates to chemical contamination of the battlefield. He pointed out that the doctrines of the United States and the Soviet Union are quite dissimilar. The Soviet Union views chemical agents as conventional weapons of mass destruction, not as instruments of escalation, and trains soldiers accordingly. The United States emphasizes the threat of retaliation to deter the enemy's use of chemical weapons. Our defensive doctrine first emphasizes contamination avoidance, followed by protection, and then decontamination. One goal is to shift chemical defense from a specialist's job to an integral part of all training in order to sustain the force and operate effectively in a chemical environment. Other points presented by MAJ Blackman include the following:

- Detectors and alarms to alert soldiers to the presence of chemicals are a top priority.
- For medical countermeasures, the order of priority would be: protection/pretreatment > antidotes > casualty treatment.

The Contaminated Battlefield in One Scenario of an Initial Attack

Battlefield (Figure 1):

10% - Percentage of the battlefield that was exposed to persistent agent (days)

50% - Percentage of the battlefield that was exposed to nonpersistent agent vapor (hours)

Personnel:

29% - Percentage of personnel that were exposed to chemical agent. Of the total troops, 10% were casualties of agent; 5% of the total force were casualties of the inappropriate use of antidotes to nerve agents

Equipment:

20% - Percentage of vehicles that were contaminated

In the field the goal will be to survive and fight with the capability of sustaining the battle for prolonged periods.

In combat, reducing/preventing morbidity and loss of duty time in the large number of casualties with less than fatal injuries is a higher priority than reducing the numbers of deaths among the small percentage of casualties that sustain potentially fatal injuries.

Approaches

Old	New
Prevent chemical casualties	Maximize combat power
Focus on individual survival	Focus on unit operations
Chemical Corps problem	Everybody's problem
Complete decontamination	Avoiding contamination and hasty decontamination (Tables 1 and 2)
Centralized decisions on MOPP levels	Decentralized, flexible decisions on MOPP levels

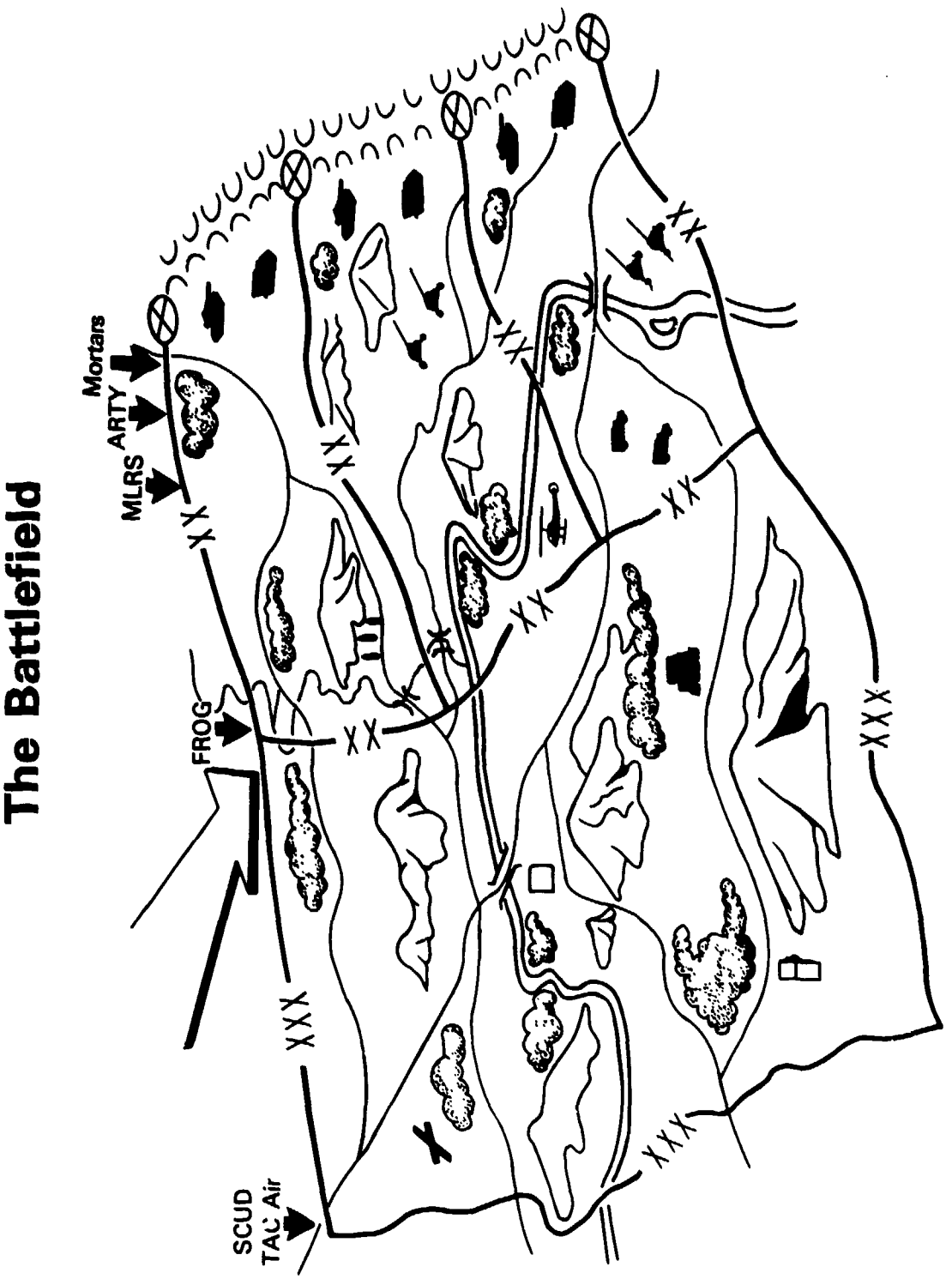

Figure 1

Table 1. Materiel Solutions--Contamination Avoidance (Chem/Bio/Tox)

Detection Function	Current	Mid-Term	Far-Term
Chem. vapor detection			
Point	Auto. Chem. alarm, M8A1	Improved Pt. Det., XM22	Mini-Pt. detectors
Remote	None	Stand-off Det. XM21	
Monitor	Det. kit, M256A1	Chem. agent monitor (CAM)	
	Water testing kit, M272	Mult. Int. Chem. agent Det. (MICAD)	All agent stand-off quantification detector/alarm
Chem. liquid detection			
Point	None	Auto. Liq. Det. (XM85/86)	Chem. agent Det. network (CADNET)
Remote	None	None	
Monitor	Det. papers, M8, M9	Improved paper	
Chem./Bio. Recon.			
On the move	None	NBC Recon.- interim	NBC Recon.- ultimate (aerial/ ground)
Biol./Tox. detection			
Alarm	None	None	Auto Biol./ Tox. Det.
Confirm			Warning confirm. Sys.
Audio/Vis. NBC alarm	None	Chem. agent Warn. Trans. Sys. (CAWTS)	
Automated NBC Info. Sys.	None	Auto. NBC Info. Sys. (ANBCIS)	

Table 2. Materiel Solutions - Protection (Individual)

Function	Current	Mid-term	Far-term
Individual			
Respirator	Mask/hood-M17,24,25	Multipurpose mask XM40, P31	
Overgarment	CPOG	Enhanced Chem. Prot. suit (ECPS)	Suit, integrated protective
	Battledress overgarment (BDO)		Full-up tactile glove
	OG-84		Full-up MULO Full-up JSOR mask
Gloves	Butyl rubber	Interim tactile gloves (7 mil/ 14 mil)	
Footwear	Booties	Interim multipurpose (MULO) boot	
Disposable barrier	None	Suit, contamination avoidance and liquid Prot. (SCALP)	
Medical Support			
Antidotes	Atropine/2-PAM-Mark I	Autoinj. Mark II replacement-amyl nitrate →	Chem. agents pretreatments, antidotes and and treatment (CAPATS)
Pretreatments	Pyridostigmine →		Anti-Rad. drugs
Front line equipment	None	Chem. patient wrap Chem. Resis. litter, bandages	Vital sign monitor, resuscitators, ventilators

MATERIAL IN VIEWGRAPHS

Integrated Battlefield Concerns

Degradation of capability caused by individual and collective protection

Restrictions on maneuver capability

Resource-intensive nature of decontamination

Lack of optimum detection and warning systems

Missions and Tasks of the Chemical Corps

Mission - Survive and sustain combat in a nuclear-biological-chemical (NBC) environment

Tasks - NBC defense

Identification/detection/warning and reconnaissance

Protection (individual and collective)

Decontamination (equipment and personnel)

- Battlefield obscuration (smoke over large area)

Contamination Avoidance

Passive - Individual protective posture

- Equipment design
- Coatings
- Covers

Active - Detectors and alarms

- Reconnaissance
- Warning

MATERIAL IN VIEWGRAPHS (continued)

Objectives for Individual Protection

Prophylaxis

Reduce performance degradation

Reduce physiological degradation

Reduce bulk and weight

Reduce resupply requirement

Skin decontamination/skin protection

Collective Protection

Permits removal of individual protective gear

Required for certain operations

Very expensive

Usually immobile

Provides islands of respite

Value increases when used repeatedly

Summary of Chemical Corps Future Objectives

Contamination avoidance

- Standoff detection systems
- Protective covers

Protection

- Nondegradative protective ensemble
- CP systems with less bulk, energy requirements and logistics burden

Decontamination

- Versatile decontamination systems
- Better decontaminants
- Nonaqueous decontamination systems

TITLE: Threat to Naval Assets and Operations

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Naval operations may be partitioned into three basic scenarios: at sea, amphibious, and fixed-shore. At sea, the major threat would be from persistent agent deposited on deck and directed toward exposed personnel. In the amphibious scenario, vesicants in persistent form would affect disembarkation operations as well as follow-on logistics and medical support. Ashore at fixed naval installations, support functions would be affected by the requirement to perform duties in full individual protective ensemble.

The effect of chemical attacks on medical support in conflicts has been of great concern to the Navy. Vesicants exacerbate the problem because of the persistent vapor hazard, which in turn requires full MOPP for protection. The effect on medical support is obvious in that casualty care functions while military personnel are so attired are significantly limited, if not prevented. Rapid detection and decontamination, therefore, become essential in order to permit reduction of MOPP levels. The medical intensity inherent in vesicant injuries will require an application of resources to a large number of casualties over a relatively short period of time. This could severely deplete treatment capability at the echelon two or three level of care. Casualty estimates would be required to better quantify such effects.

THREAT TO NAVAL ASSETS AND OPERATIONS
Presented by CAPT W. M. Parsons, MSC, USN

CAPT Parsons discussed the vesicant threat to naval operations. Three basic scenarios were presented, i.e., at sea, amphibious, and fixed-shore. The impact of chemical attacks on medical support in conflicts and the Navy's concerns were presented. Some of the concerns and needs included the following:

- The Navy has unique problems with regard to vesicants that are a combination of the problems faced by the other services.

- Navy planners anticipate that there would be some warning that would enable them to prepare for an imminent threat.

- Primary naval targets include: (1) logistics and support bases, e.g., ports, ship/aircraft repair facilities, and air station ; (2) close inshore vessels (in an amphibious operation); and (3) amphibious ground forces and supporting shore-based units.

- Naval concerns regarding vesicants are that: (1) they are casualty-producing; (2) they persist and are difficult to detoxify; (3) they have latent effects; (4) they tax medical resources heavily; and (5) they prolong encapsulation.

- The Navy intends to handle vesicant casualties in a manner similar to that for other contaminated casualties. By doctrine, the casualties must be decontaminated before treatment by health care providers is started.

- The use of vesicants could produce big problems for the Navy, particularly if personnel are forced to go into MOPP-4.

- With one exception, fleet hospital ships have no collective protection.

- Naval requirements/needs include: (1) detectors; (2) decontaminants that are safe and rapid; (3) barrier creams; and (4) a definitive determination of "How clean is clean?"

TITLE: Impacts of Vesicants on Air Force Operations

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The U.S. Air Force operates its tactical combat forces in each of three theaters of operations: Europe, the Pacific, and the world-wide deployable forces. A common feature of Air Force operations in any theater is the dependence upon fixed operating sites (runways) and the resultant high value placed upon these locations by opposing forces. Because of the probability of chemical warfare being directed against high-value targets, the Air Force must be prepared and protected to survive and fight in a chemically contaminated environment.

The use of persistent vesicant chemical warfare agents is anticipated against Air Force targets. Their projected use places additional burdens of protection, decontamination, and medical care upon Air Force operators over and above those associated with chemical warfare nerve agents. Impacts are clearly seen in requirements for vesicant-unique doctrine, training, logistics, and medical care.

The goal of the Air force is to reduce the limitations placed upon fixed-site operations caused by persistent vesicant contamination. This can best be accomplished by the development of protective skin barriers, effective antidotes/treatment regimes, and personnel and equipment decontaminants.

IMPACT OF VESICANTS ON AIR FORCE OPERATIONS
Presented by Lt Col Gary R. McNutt, USAF, BSC

Lt Col McNutt's presentation defined the Air Force assessment of the effects of vesicant contamination. Because of the Air Force requirement for fixed operating sites (runways), the service has different problems from the other service branches. Problems/needs presented included the following:

- The Air Force operates in three theaters of operation: Europe, the Pacific, and the worldwide deployable forces.
- The Air Force is dependent on fixed operating sites (airfields, munitions storage, and bare base locations), which are expected to have high value placed on them by opposing forces.
- The high probability of chemical warfare directed against high value targets requires the Air Force to be prepared and protected so that individuals can survive and fight in a chemically contaminated environment ("fighting dirty").
- The U.S. Air Force program for chemical warfare defense includes personal protection and collective protection.
- The personal protection program covers MOPP 4, the MCU-2P mask, and the unique aircrew ensemble.
- The collective protection program includes chemical overscope facilities, survivable collective protection shelters, contamination control area procedures, and resource protection (TAB V shelters). Low-level, long-term exposures are expected in collective protection shelters.
- There is a clear need for unique vesicant doctrine, training, logistics, and medical care.
- To best reduce the limitations caused by persistent vesicant contamination on fixed site operations, the Air Force needs to develop: (1) effective topical barriers, (2) effective antidotes/treatment regimes, (3) personnel and equipment decontaminants; and (4) mustard and lewisite detectors/alarms.

MEDICAL CONSIDERATIONS AND IMPLICATIONS

Presented by Robert H. Mosebar, M.D.
Academy of Health Sciences,
Fort Sam Houston, TX 78234-6100

Dr. Mosebar discussed medical considerations relevant to vesicant exposure, citing information from World Wars I and II and the Iran-Iraq conflict. Many areas of medical management were examined, with emphasis placed on the difficulties of triage, the low incidence of mortality, and the high number of casualties requiring hospitalization with long-term, often intensive nursing care. Also noted were the lack of specific and effective treatment regimens and of personnel experienced in treating vesicant casualties. Some specifics of the presentation included the following:

- Americans in World War I were not adequately prepared for the chemical threat to which they were ultimately exposed. Fifty percent of all U.S. Army hospitalized patients in World War I were chemical agent casualties.

- Lesions produced by mustard heal slowly.

- Mortality due to mustard exposure is very low (<2%); however, length of hospitalization due to mustard exposure is often prolonged (>30 days).

- In World War I, an ointment called Sagpaste, which contained chlorine (among other things) in a vaseline base (exact formula not known), was effective in preventing mustard burns, in alleviating the pain associated with mustard burns, and in exterminating body lice. However, if Sagpaste was not removed shortly after contamination by mustard, it became a hazard itself, resulting in even more serious injuries.

- There are three broad categories of mustard blister casualties: (1) those with minor burns/blisters that present little problem to medical personnel and do not prevent return to duty; (2) casualties having burns/blisters of sufficient degree to require hospitalization; and (3) between these two categories, a large group of casualties that will create major medical problems. Some in this middle group, as their injuries develop progressively, may require two or more trips for clinical evaluation before final disposition is possible.

- The question was raised whether separate field hospitals for chemical casualties should be set up as in World War I. During World War I, one-fourth of the hospital beds were allocated specifically to gas casualties.

- To date, all medications to prevent blister formation have been ineffective.

- Once blistering has occurred, the best treatment available is symptomatic (i.e., for pain, itching, and dehydration).

- Prevention of infection is also a major consideration.

- In 233 Iranian casualties of the Gulf War, the most common clinical symptoms were as follows: respiratory (95%), ophthalmologic (92%), cutaneous (83%), central nervous system (83%), gastrointestinal (68%), and cardiovascular (58%).

- Because of eye involvement, an ophthalmologist should be at a hospital in the Corps zone.

- The major complication in terms of treatment of mustard casualties is the nursing problem. Many of the most severely injured require one-on-one nursing care for 24 hours a day.

- Mustard acts by affecting DNA.

- Skin repair and growth are very slow because of DNA damage. Nevertheless, grafts are rarely needed because the injury only extends into the superficial dermis. There is an intermediate stage of healing, frequently lasting a month, during which friable granulation tissue persists. It bleeds with minimal trauma and is easily infected, so the soldier cannot return to duty.

DISCUSSION

Session II

The treatment of mixed traumas, i.e., conventional wounds plus agent injury, was the first topic discussed. Attempts to develop guidelines for these injuries are ongoing, but are inconclusive thus far. Treatment of conventional trauma is based on historical information, whereas information relating to the vesicant threat is being derived from current models. At present, there are no established guidelines for triage and prognostication. In addition, the information available at this time does not allow for quantitative evaluation of vesicant injuries, making mixed trauma predictions difficult to impossible. Dr. Papirmeister mentioned the proposed development of a quick test involving the alkylation of hemoglobin to quantitate a casualty's mustard exposure.

The duration of effectiveness of MOPP suits and their deployment were discussed. The camouflage battle dress overgarment was stated to be effective for 22 days (up to 30 days with some increased risk). It will provide 24 hours of protection following contamination. The Army issues three suits per soldier, with additional suits in reserve.

An additional problem in protection concerns the hands of soldiers, pilots, medics, and others requiring fine motor control. The Air Force and Army intend to issue butyl rubber gloves of varying thickness, ranging from 25 mm for the thickest glove to 7.14 mm for the thinnest. Choice of glove thickness is determined by the individual's requirement for tactile sensitivity. The Air Force has a glove of 14-mm thickness. The durability of the glove is inversely related to the thickness. The Army has tested butyl rubber gloves worn by corpsmen performing activities they would be doing in the field under combat conditions (i.e., starting intravenous infusions, putting on splints, and dressing wounds) and has found that corpsmen were able to adjust to the gloves and could adequately perform these tasks after a week of training and practice.

Mustard injuries have been reported to heal more slowly than thermal burns by physicians in Belgium, Germany, and London who have treated mustard injuries. This inference was drawn from testimony of burn ward personnel and was not based on well-controlled studies.

The nature of mustard injuries raises questions about evacuation. The policies regarding evacuation of mustard casualties with hand and eye injuries, particularly chemically induced "blindness," were discussed. With respect to hand injuries, the Army views the need to return to duty as an issue of such extreme urgency that, in the Army environment at least, it is anticipated that the casualties will be returned to duty even with bandaged hands unless this would be permanently injurious to the individual. It is likely that two-thirds of eye casualties, after being seen by an ophthalmologist, will be sent back to duty within 2 or 3 weeks.

It is desirable to develop models of numbers and types of casualties that can be expected in a chemical environment. At this time, however, there is very little information readily available to the military physician; this is an issue that must be addressed. Dose-response predictions for vesicants are currently being developed under a USAMRDC contract with the MITRE Corporation.

The use of extended-wear contact lenses in a CW environment was discussed. Contact lenses will exacerbate vesicant eye injuries because they trap agents and prolong contact of chemicals with the cornea. There is a European study suggesting that certain kinds of lenses do offer some limited protection against certain agents. The Air Force is under tremendous pressure from its aircrews to allow contact lens use; however, to date, doctrine forbids them.

The discussion was summarized by MAJ Daniel Rickett:

We have demonstrated that a vesicant threat exists. We do not have quantitative information on exactly what that hazard is relative to other sources of battlefield injury. We do not have good methods for estimating how many casualties we might expect, whether they will have combined injuries, or what percentages of injuries will be seen--the answers are driven by specific scenarios. It is interesting that in the discussion sessions we have focused on protection, i.e., physical protection. We may be heading toward the concept of a lightweight suit that gives personnel time to get into a definitive suit. It must be remembered that the medical community, at least the USAMRDC side of it, has to work on those things that we employ when protection fails--in the case of decontaminants, when avoidance attempts fail.

Session III

Deficiencies in the Defense Against Vesicants

THE TRADOC COMBAT DEVELOPER'S PERSPECTIVE

Presented by MAJ Merrill S. Blackman, CM
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AV 865-3877

Assessment of needs:

Contamination avoidance:

1. Standoff detection-alarm systems (for reconnaissance and monitoring persistence of surety agents)
2. Protective covers

Protection:

1. A nondegradable protective ensemble
2. A collective protection system with less bulk, lower energy requirements, and a smaller logistics burden
3. Decontamination systems that are logistically less burdensome, i.e., require minimal water, charcoal, and manpower

Summary:

1. There is a need for protective equipment and decontamination systems that are not tied to charcoal or water.
2. The decrement in the efficacy of protective overgarments when challenged with small particle (approx. 1 um) aerosols in wind conditions of 5 m.p.h. should be addressed.

DEFICIENCIES IN THE MEDICAL RESPONSE

Presented by Robert H. Mosebar, M.D.
Academy of Health Sciences
Fort Sam Houston, TX 78234-6100
AV 471-7130

Dr. Mosebar discussed the areas in medical treatment of chemical casualties that need improvement. No definitive document covering this subject has been produced; however, a field manual is currently in production. There is a need for a decontaminant that is effective, can be stored in a small container, will cover a large area, and is safe to use in wounds and around the eyes. Decontamination personnel requirements need to be addressed, i.e., who will perform decontamination of patients before they receive medical attention? There are many unsolved problems inherent in the medical response to the chemically injured soldier.

Specific points addressed include the following:

- The current decontamination kit M258A1 contains chemicals that are toxic to skin, e.g., phenol, sodium hydroxide, and ammonia.
- The quantity of water required for decontamination systems is too great. Also, cold water on a patient in shock would be detrimental, but it would be difficult, logistically, to provide heated water.
- Medics cannot be spared from their regular duties to perform decontamination. Perhaps cooks, bakers, and others can be trained to decontaminate chemical casualties and to send the "clean" patients to the medics. Patient/medical personnel must be "clean" before entering the collective protection facilities.
- Needs:
  1. Reduced requirement for intensive medical care
  2. An antidote that can be be carried by the soldier
  3. Pretreatment (preferably long-term)
- Current triage requirements are difficult for medical personnel; i.e., delaying the treatment of the severely wounded in order to tend less severely wounded, salvageable patients deeply offends medical instincts.

Session IV

Current Medical Research

TITLE: Mechanism of Action of Sulfur Mustard

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In spite of intensive research on the mechanism of mustard-induced injury during the past seven decades, the precise cause and pathogenesis are only now beginning to be unraveled. Although many studies at the molecular and cellular levels have identified DNA as an especially HD-sensitive target, alkylating damage to other cellular sites (such as the membrane network, mRNA-mediated protein synthesis, sensitive structural proteins, and enzymes) has not been unequivocally excluded from consideration.

The most compelling supporting evidence that DNA is the cellular target primarily responsible for initiating HD toxicity is: (a) the formation of an HD-DNA adduct that cross-links the complementary DNA strands, with only a small number of such cross-links being sufficient to interfere with replication of the genetic material and cell division; (b) the formation of unstable monofunctional DNA adducts, which results in both spontaneous and enzymatic production of DNA breaks with genotoxic and cytotoxic consequences; (c) the formation of low frequency DNA adducts that have a high mutagenic/carcinogenic potential; (d) the identification of several DNA repair processes that are able to excise structural and mutagenic DNA defects and restore a fully functional genome; and (e) the discovery of mutant cells that are incapable of repairing specific DNA damages and therefore are extremely sensitive to mustards. The ability of DNA repair inhibitors to exacerbate the severity of the cutaneous HD injury demonstrates that the formation of DNA damage is also an initiating event that is causally related to the later development of pathologic changes.

More recently, a biochemical hypothesis for the HD injury to human skin was proposed that links DNA damage to alterations of metabolism and the delayed development of pathologic changes. According to this hypothesis, breaks produced at apurinic sites in HD-treated DNA stimulate the activity of the chromosomal enzyme poly(ADP-ribose) polymerase (PADPRP). This enzyme uses NAD^+ as a substrate, and vesicating doses of HD (i.e., $\geq 10^7$ alkylations/genome) would be sufficient to cause almost a complete depletion of the NAD^+ content in epidermal keratinocytes. Such depletion of this vital cofactor then would trigger the following sequelae: inhibition of glycolysis, stimulation of the hexose monophosphate shunt, release of proteases, loss of membrane integrity, cell necrosis, loss of epidermal/dermal adhesion, increase in osmotic pressure, accumulation of edema fluid, and development of subepidermal blisters. According to this hypothesis, vesication is attributed to monofunctional rather than bifunctional alkylations in DNA. The potency of several monofunctional sulfur mustards as vesicants is consistent with this view. However, the characteristic slow healing process of the cutaneous HD injury is thought to be due to the presence of cytocidal cross-links in adjacent basal cells.

Biochemical and morphological evidence was obtained at the molecular and cellular levels and in human skin grafted to athymic nude mice that supports the validity of the hypothesis. The HD-induced loss of skin NAD^+ levels, preventable by PADPRP inhibitors, was found to take place during the asymptomatic latent period and correlated with the severity of the subsequent injury. Such early biochemical events are of interest because they provide the greatest potential for therapeutic exploitation.

MECHANISM OF ACTION OF SULFUR MUSTARD: A BIOCHEMICAL HYPOTHESIS

Presented by Bruno Papirmeister, Sc.D.

The precise cause and pathogenesis of mustard-induced injury have only recently begun to be unraveled. Dr. Papirmeister presented evidence that DNA is the most critical cellular target. Nevertheless, alkylating damage--particularly at high doses--to other cellular sites (such as membranes, mRNA-directed protein synthesis, and structural and enzymatic proteins) has not been unequivocally excluded from consideration. Evidence was shown that DNA damage is an initiating event that is causally related to the later development of skin lesions. A biochemical hypothesis was presented that links DNA alkylations in basal epidermal cells to an inhibition of cellular energy metabolism and the delayed development of blisters. This hypothesis, which has been validated in part in human skin grafts on athymic nude mice, accounts for the early events occurring during the latent period that precedes blister formation. Such early metabolic derangements lend themselves to manipulative exploitation and provide novel and rational approaches for therapeutic intervention. A similar molecular mechanism is probably involved in the killing of other mustard-sensitive cells and tissues (e.g., human lymphocytes).

Reactions of mustard with cellular targets:

- Reactions of mustard in aqueous media occur as a two-step process: (a) a positively charged, cyclic sulfonium intermediate is formed; and (b) there is a rapid reaction of the sulfonium intermediate with negatively charged groups, the extent of alkylation being determined by the concentration and avidity of the particular group (Figure 1).

- Since the bifunctional mustard has two reactive centers, it has the ability to cross-link target molecules (e.g., the N7G-HD-N7G DNA adduct).

- Although mustard extensively alkylates nucleic acids, proteins, and a variety of metabolites, strong evidence implicates DNA as the most critical cellular target. Cells incapable of repairing mustard-damaged DNA are more sensitive than are their repair-capable counterparts.

- Only a few interstrand cross-links in DNA suffice to inhibit replication and cell division. Monofunctional mustard adducts in DNA are responsible for the mutagenic/carcinogenic properties of mustard, and can also initiate the formation of apurinic sites and the production of DNA breaks. The excision of interstrand crosslinks produced by nitrogen mustard in Chinese hamster ovary cells is shown in Figure 2.

Biochemical hypothesis for the cutaneous mustard injury:

- The ability of DNA repair inhibitors to exacerbate the mustard-induced skin injury supports the hypothesis that DNA damage also plays a critical role in vesication.

- A recent biochemical hypothesis links mustard-induced DNA damage to metabolic disturbances and the delayed development of pathologic changes (Figure 3).

- It is postulated that breaks produced at apurinic sites in the DNA of epidermal cells activate a chromosomal enzyme, poly(ADP-ribose) polymerase (PADPRP), which uses NAD^+ as a substrate. At vesicating doses, there is almost a complete depletion of NAD^+ that results in the inhibition of glycolysis, loss of cellular energy supply, cell necrosis, protease release, loss of epidermal/dermal adhesion, increase in osmotic pressure, fluid accumulation, and formation of subepidermal blisters (Figures 4 and 5).

- Vesication is attributed to monofunctional rather than bifunctional alkylations in DNA. Consistent with this view is the high vesicant potency of several monofunctional sulfur mustards. However, the presence of cytocidal crosslinks in the DNA of adjacent basal cells may retard the healing of cutaneous mustard lesions.

- The biochemical hypothesis has been validated in part by *in vitro* studies with isolated DNA (Figure 6) and cultured cells and by *in vivo* studies using athymic nude mice bearing human skin grafts. In the latter, dose-dependent losses of NAD^+ were observed during the latent period (Figure 7). These NAD^+ losses were attenuated by PADPRP inhibitors (Figure 8) and were correlated with the severity of the subsequent injury (Figure 9).

- Knowledge of the biochemical events occurring during the latent period after the fixation of mustard are of interest because they provide the greatest potential for therapeutic exploitation.

eactions of Mustard Gas with Cellular Targets, H_2O, Detoxicants

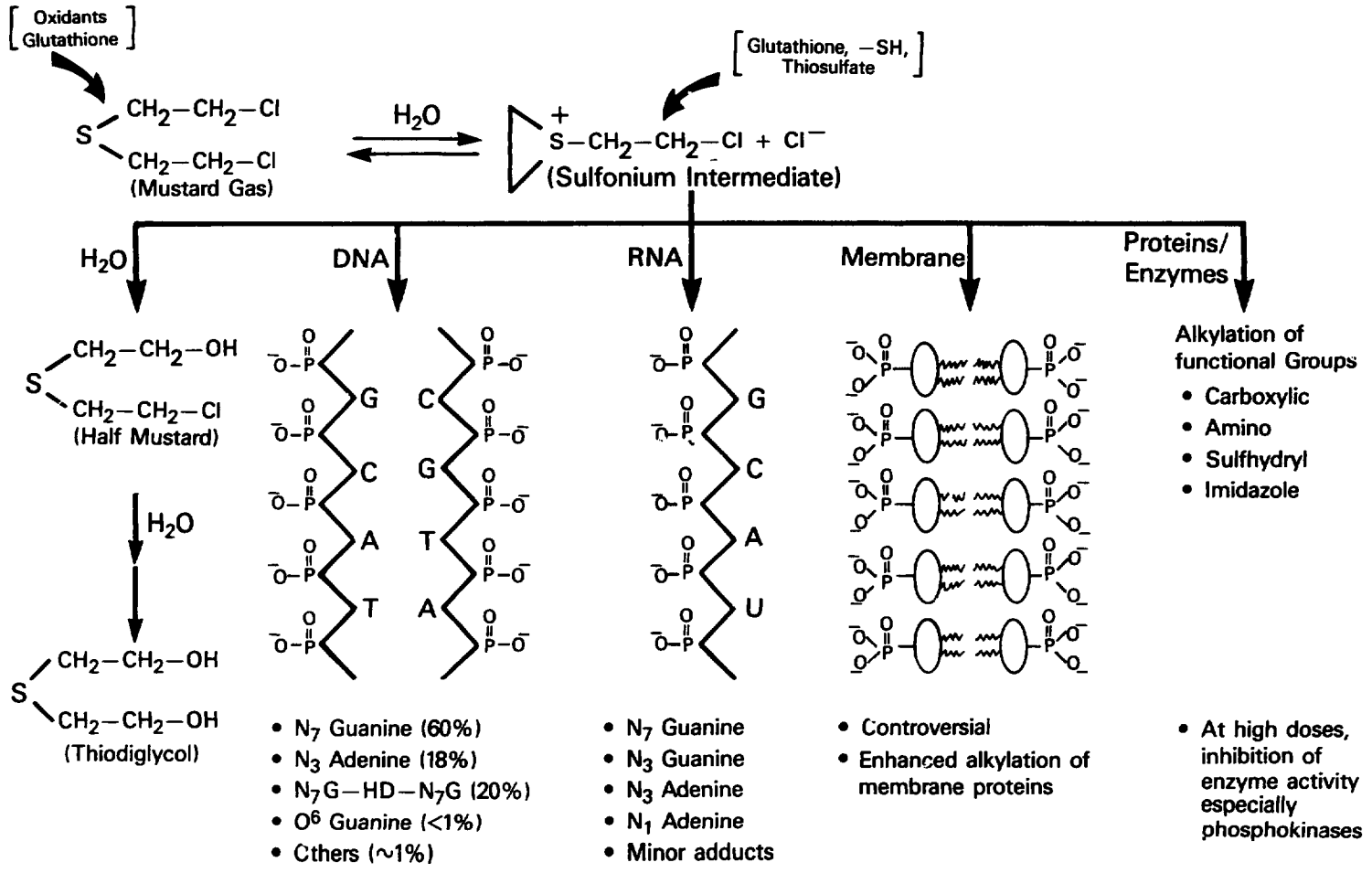

Figure 1. The reaction sequence of mustard in aqueous media is shown. The rate-limiting step is the reversible formation of a positively charged cyclic sulfonium intermediate which then rapidly reacts with negatively charged cellular targets, the extent of alkylation being determined by the concentration and avidity of the chemical group involved. Potential sites for detoxification of mustard are shown by heavy arrows.

Mustard Induction of DNA Cross-links and Their Repair

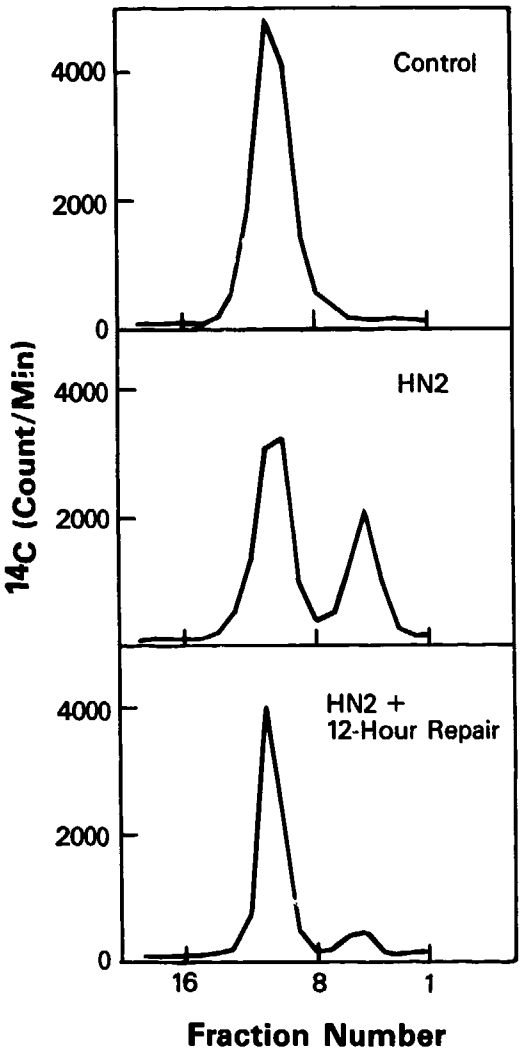

Figure 2. ^{14}C-labeled Chinese hamster ovary cells were incubated for 30 minutes in medium containing 3 x 10^{-5} M nitrogen mustard (HN2). The cells were either harvested immediately or incubated for 12 hours in fresh medium. Following denaturation and renaturation, the DNA was analyzed on CsCl gradients at pH 11. (Adapted from Clarkson and Mitchell 1981)

Biochemical Hypothesis for Cutaneous HD Injury

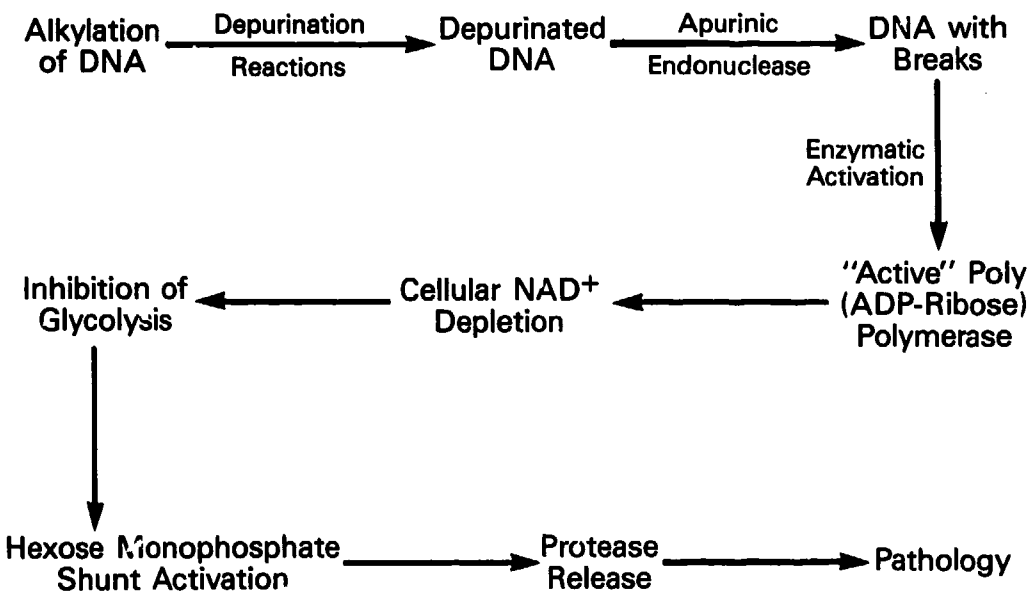

Figure 3. The proposed mechanism describes sequential events which link DNA alkylation to metabolic disturbances and the development of pathology. (Adapted from Papirmeister et al. 1985)

Pyknosis in Basal Keratinocytes Due to HD Expcsure of Human Skin Grafts in Athymic Nude Mice

Figure 4. (A) Light micrqscopic changes in facial skin graft 12 hours after exposure to HD, 635 ug/cm^2. Note the focal appearance of pyknotic nuclei, especially in the basal cell layer. Some of the suprabasal keratinocytes have become hypochromic. (hematoxylin-eosin, 400X; adapted from Papirmeister et al. 1984a) (B) Pyknotic basal$_2$nucleus (n) 24 hours after exposure of human fore-skin graft to HD, 60 ug/cm^2. Note decrease of euchromatin, condensation of heterochromatin, extensive damage and blebbing of the nuclear membrane (arrows), and formation of debris-filled perinuclear and cytoplasmic vacuoles (v). The nuclei of neighboring basal cells show less damage. (Adapted from Papirmeister et al. 1984b)

Microblisters in HD-Treated Human Skin Grafts in Athymic Nude Mice

Figure 5. (A) Light microscopic changes in facial skin graft 48 hours after exposure to HD, 635 ug/cm^2. Note complete separation of the epidermis from the dermis just above the epidermal-dermal junction with formation of a subepidermal blister cavity. (Humphrey stain, 16UX; adapted from Papirmeister 1984a) (B) Ultrastructural changes in microblister in human facial skin graft 48 hours after exposure to HD, 635 ug/cm^2. Note that cavity was formed by breakage of anchoring filaments (af) from their attachment sites on the basal lamina (bl), which forms the base of the blister. Intact hemidesmosomes remain attached to the damaged basal cell plasma membrane, which forms the roof of the blister. Intracellular basal cell debris (arrows) extrudes into the blister (b) cavity. The adjacent dermis (d) appears well organized. (Adapted from Papirmeister et al. 1984b)

Production of DNA Breaks by Treatment with Half Sulfur Mustard

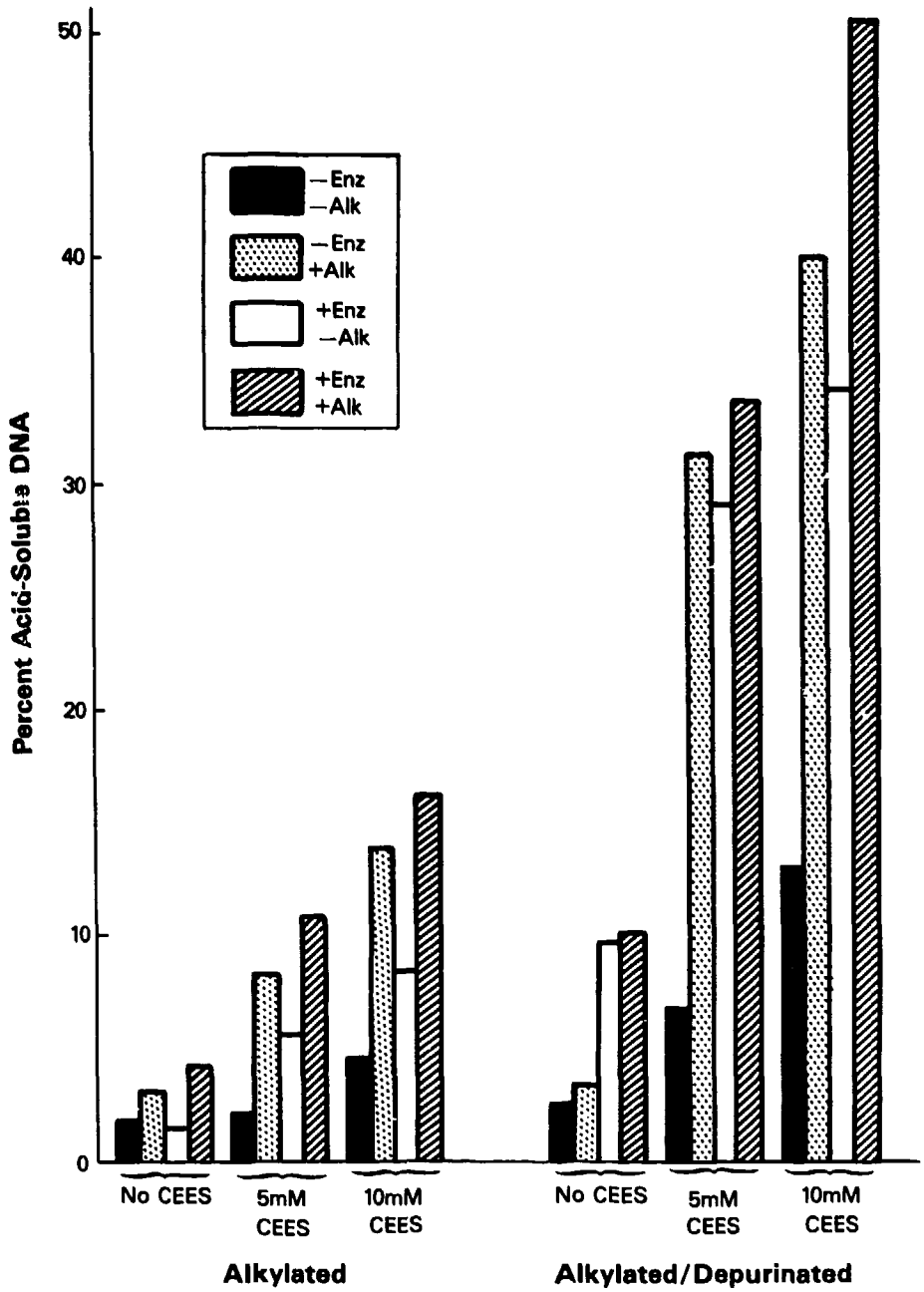

Figure 6. [^{3}H]Thymidine-labeled sonicated E. coli DNA was treated with half sulfur mustard (CEES) at 5 and 10 mM. Half of each preparation was incubated for 48 hours at 37°C to provide alkylated/depurinated samples. Unalkylated controls were handled in the same manner. All preparations were incubated for 30 minutes at 37°C with purified apurinic endonuclease from E. coli. Following incubation, preparations were subjected to filtration on Sephadex G-15, and the fractions excluded by the gel were pooled. Duplicate aliquots were then either precipitated with perchloric acid directly or following a 15-minute incubation at 37°C with 0.2 N NaOH, and the percentage of the radioactivity rendered acid-soluble was determined. The bar graph compares degradation produced by the apurinic endonuclease with that resulting from cleavage of alkali-labile sites. (Adapted from Papirmeister et al. 1985)

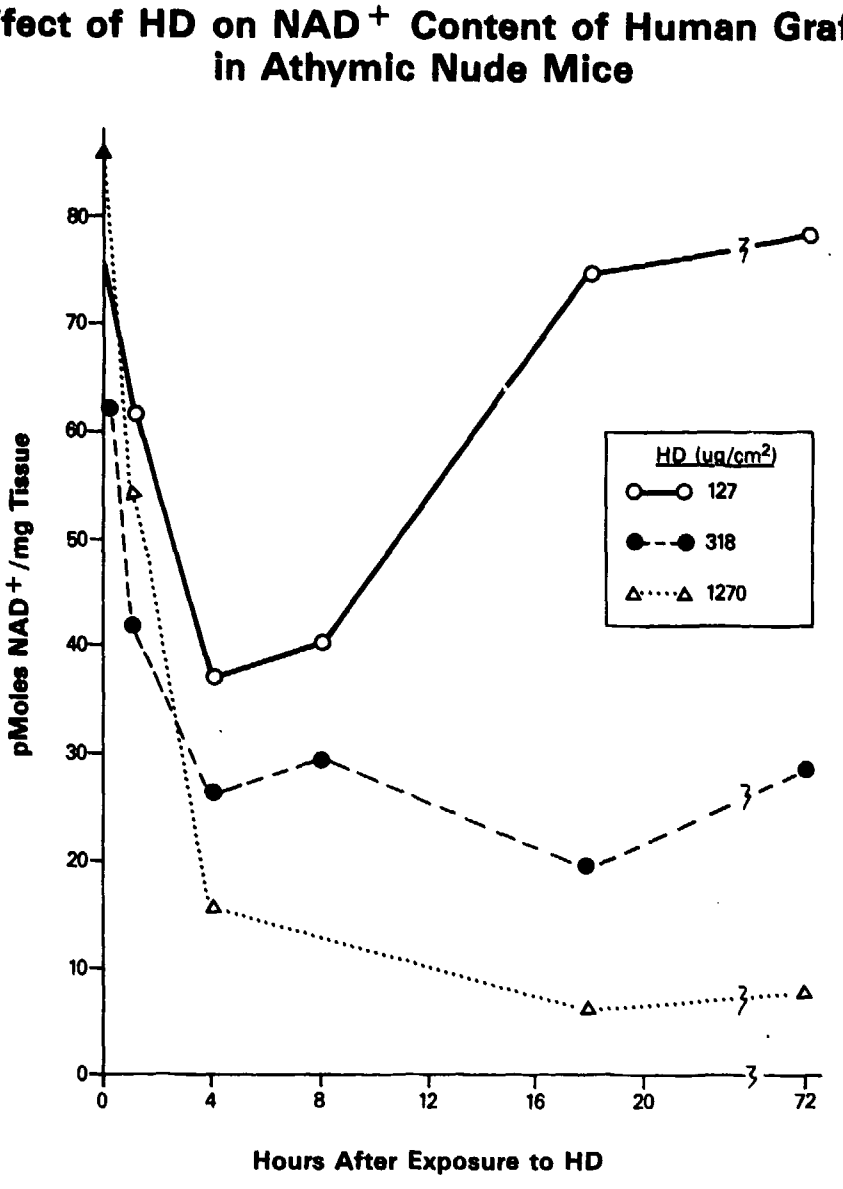

Figure 7. Grafts were exposed to varying concentrations of HD. Animals were sacrificed at the designated postexposure times, skin grafts were removed, and 4-mm biopsy punch skin samples were taken immediately. The skin samples were quickly frozen in liquid nitrogen, weighed, and then extracted twice with 0.5 M $HClO_4$ overnight at 4°C. After neutralization with KOH and removal of insoluble $KClO_4$, the supernatants were pooled and assayed for NAD^+, using the enzymatic cycling assay. Each time point was the average of two separate skin punches assayed in triplicate. (Adapted from Papirmeister et al. 1985)

Effect of a Poly(ADP-Ribose) Polymerase Inhibitor on HD-Induced NAD$^+$ Loss in Human Skin Grafts in Athymic Nude Mice

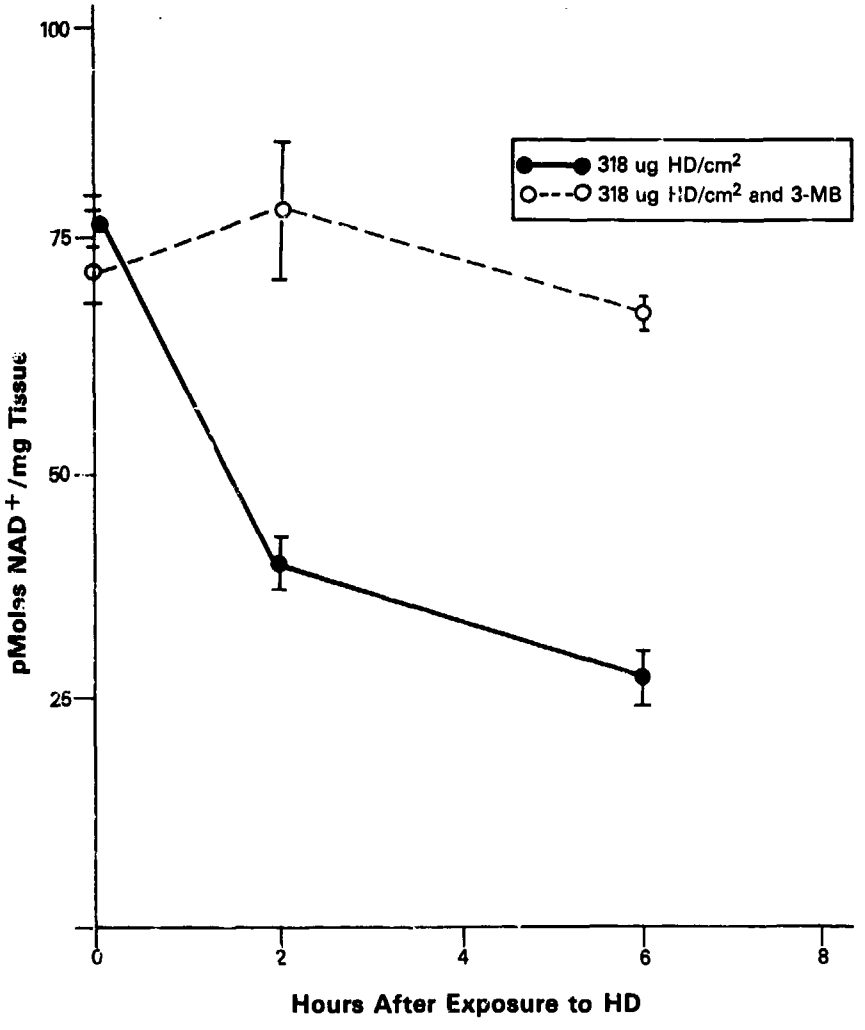

Figure 8. The grafted mice were injected with 3-methoxybenzamide (3.75 mg, i.p.) 1 hour before exposure to HD. Human skin grafts were exposed to HD and mice were sacrificed at the designated times. The skin samples were extracted as described for Figure 7. Error bars represent the standard error of the mean for three biopsy skin punches (measured in duplicate) from each animal. (Adapted from Papirmeister et al. 1985)

Relationship Between "Fixed" HD, NAD$^+$ Content, and Severity of Skin Injury

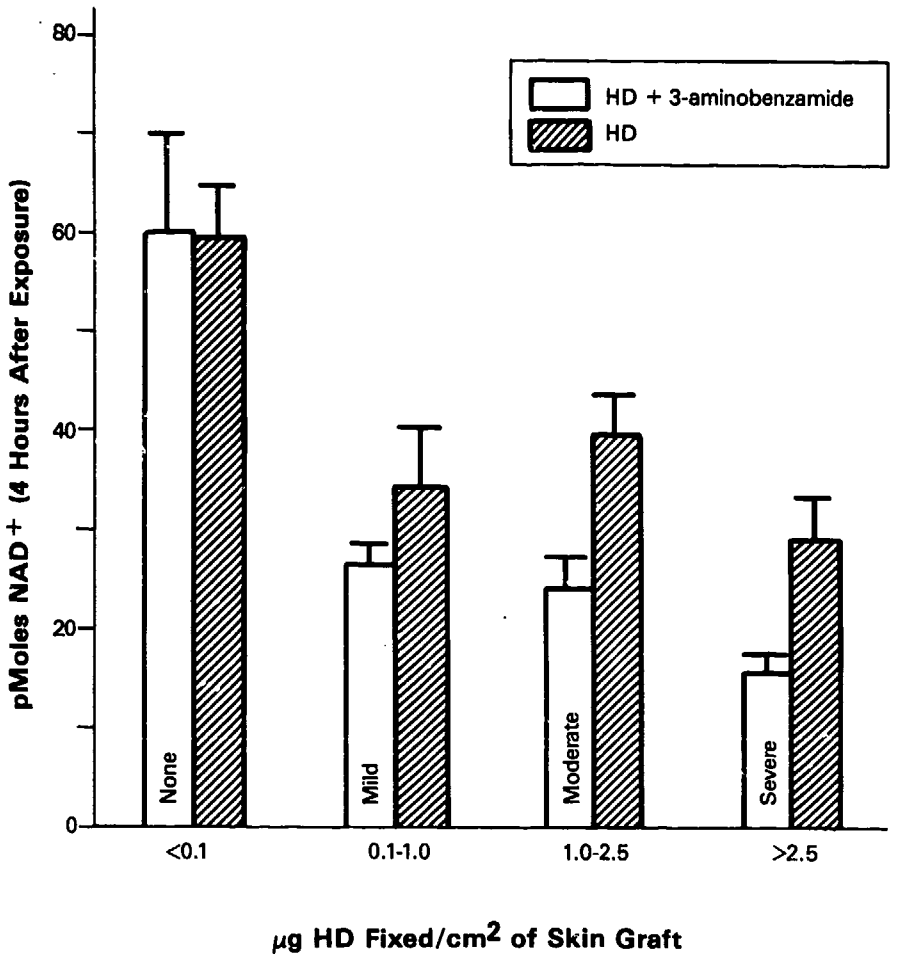

Figure 9. Athymic nude mice were treated with either 3-aminobenzamide (50 mg/kg, i.p.) or vehicle for 30 minutes before exposure of human grafts to varying concentrations of ^{14}C-labeled HD. Animals were given an additional injection of 3-aminobenzamide (50 mg/kg, i.p.) 90 minutes after exposure to HD. The amount of HD fixed/cm^2 of human skin graft was correlated with the severity of skin damage previously described in humans (Renshaw 1946). Nude mice were sacrificed 4 hours after exposure, skin grafts were removed, and 4-mm biopsy punch skin samples were taken immediately. The skin samples were processed as for Figure 7. The skin biopsy punches were combusted in a Packard Tri-Carb Sample Oxidizer and the "fixed" radioactivity contained in these punches was determined by scintillation counting. (Adapted from Papirmeister 1985)

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TITLE: Development of a Safe and Effective Skin Decontamination System - A Program Update

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Skin decontamination plays an important role in chemical warfare (CW) defense. A large number of decontaminating systems and methods have been studied for the removal and/or destruction of nerve and blister agents. Unfortunately, most chemical approaches were developed for equipment decontamination and are too corrosive and hazardous for use on skin. In general, there is no single universal skin decontamination system currently available that is both safe and effective for decontaminating nerve agents (e.g., soman, sarin, VX) and blister agents (e.g., distilled mustard, lewisite). The existing U.S. skin decontamination system is the Personal Decontamination Kit, M258A1. The M258A1 kit contains chemicals that are highly irritating to skin and are hazardous to the eyes. A separate Training Aid (M58A1) is required because of the toxicity of the M258A1 kit.

The current development objective is to produce and field a skin decontamination system that possesses a decontamination capacity superior to the M258A1 kit, is nontoxic and nonirritating, is effective against multiple chemical agents and toxins, and is safe for training use.

Under a U.S. Army Medical Research and Development Command contract, formulations containing ion exchange resins and synthetic absorbents (available from Rohm and Haas Company) were shown to be safe and effective as skin decontaminants for CW defense. In addition to the types of activities one expects in this development effort (i.e., toxicology, efficacy evaluation, formulation/packaging development, etc.), operational aspects had to be addressed. Prototype resin-based skin decontamination kits in a novel "soft pack" configuration were tested by the U.S. Army Armor Engineer Board to determine user acceptance and operational effectiveness compared to the M258A1 system.

The current skin decontamination program is in the Demonstration and Validation phase of development. A Milestone I/II In-Process Review is scheduled for March 1987. Its purpose is to evaluate the progress to date and, if the project results justify, recommend entry into Full-Scale Development.

Factors that drive the design and formulation of the resin components, kit configuration, and application use procedures are discussed in this presentation. Skin decontamination doctrine, current capability, product development and testing, and a summary of the program status are discussed.

This work is supported in part by the U.S. Army Medical Research and Development Command under Contracts DAMD17-83-C-3071 and DAMD17-85-C-5200.

DEVELOPMENT OF A SAFE AND EFFECTIVE SKIN DECONTAMINATION SYSTEM - A PROGRAM UPDATE

Presented by LTC Donald G. Harrington, VC

LTC Harrington presented a program update of formulations containing ion exchange resins and synthetic absorbents that have been shown to be safe and effective as skin decontaminants and are being tested for inclusion in a decontamination kit for the individual soldier, to replace the M258A1 Personal Decontamination Kit and M58A1 Training Aid. The factors that affected the design and formulation of the resin components, the kit configuration, and the application use procedures were discussed. Highlights were skin decontamination doctrine, current capability, product development and testing, and a summary of the program status.

Specific points presented include the following:

- Development of a replacement kit is a joint services effort.
- The M258A1 is a two component system, one of which contains glass ampules, thereby requiring packaging in a hard case.
- Basic limitations of the current kit were identified:
  1. The contents are toxic.
  2. The contents are irritating to skin and pose a significant eye contact hazard.
  3. The kit is difficult to use in MOPP gear.
  4. Because of the toxicity, a separate training kit is required.
  5. The liquid components cannot meet some of the cold storage requirements (i.e., -60°F).
  6. The kit contains flammable liquids and therefore cannot be shipped in pallet-sized quantities by military airlift.

- The resins have these advantages:

  1. They are nontoxic and nonirritating (they can be used around eyes and wounds without any significant problems).

  2. They are effective against nerve agents and vesicants (they sorb nerve agents or vesicants and then detoxify them over time).

  3. There is no off-gassing problem.

  4. The delivery kit is a soft pack containing six packets, which will provide twice the number of skin decontamination applications as the M258A1.

  5. New resins (there are ongoing studies of second- and third-generation resins) can easily be incorporated into the kit.

- The resin base concept was transitioned from ICD to USAMMDA for development in 1985.

- In October 1986, after receiving new information, the FDA reclassified resins as noncontrolled substances for DoD application in skin decontamination.

- The Milestone II In-Process Review (IPR) that is scheduled for March 1987 will determine the readiness to enter into full-scale development.

- The Milestone III IPR scheduled for late 1989 will give authorization for production.

- Out of six resin decontamination systems investigated, two have been shown to satisfy safety and decontamination requirements:

  1. Ambergard XE-555

  2. Ambergard XE-556

- The decontamination procedure of choice for the T2 mycotoxin is soap and water; however, in the battlefield scenario, the resin-based system may afford a significant degree of efficacy against T2 toxin through physical removal.

- In customer operational tests (six soldiers at Fort Knox applied the kit; fifty soldiers at Fort Bragg carried the kit for over a week):

  1. Seven out of eight critical test issues were met. Comments from the users were favorable and emphasized ease of use and satisfaction with the soft pack concept.

  2. One shortcoming was noted. Several soldiers reported that loose resin powder caused a slight dusting effect under the mask during facial decontamination. Corrective action to remedy the problem is under way.

Summary: It is likely that the resin base concept will replace the M258A1, although there will probably never be a "golden bullet" or a single universal skin decontaminant that is optimal for all threat agents.

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TITLE: Animal Models for Vesicant-Induced Skin Injury

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Attempts to study the effects of vesicants on laboratory animals have been hindered by the inability to adequately reproduce the injury seen in human skin, particularly vesicles and bullae. Furthermore, the structure of human skin is unique and quite variable from one area of the body to the next. These differences in cutaneous morphology (and presumably function) are clinically relevant and need to be accounted for in the study of vesicating agents.

The grafted human skin/nude mouse model is an attempt to circumvent many of the problems associated with using laboratory animals as models for human skin injury. The nude mouse is an athymic mutant that has a severely impaired cellular immunity as well as a paucity of epidermal appendages. Athymic nude mice will accept xenografts from many other species, including humans. Split-thickness and thin full-thickness human skin has been grafted successfully in our laboratory. Approximately 70% of the grafts take, and the transplants generally survive for the life of the mouse, which can be up to 6 months. Grafted human skin retains the histological identity of its donor and will even grow hair if the pilar-sebaceous apparatus is included in the graft. The grafted human skin/nude mouse model has proved to be particularly valuable in evaluating the effects of highly toxic substances on human skin. For example, both sulfur mustard and arsenicals have been studied on human skin grafts, and the histological and clinical alterations of the exposed grafts mimic, to a large degree, those that are seen with intact human skin. The disadvantages of the nude mouse model relate primarily to the vulnerability of the animal, the small surface area of grafted tissue, and the attenuated inflammatory response (secondary to altered T-cell function).

The cyclosporine-treated nude rat is an improvement on that system and may offer new ways to study the pathological changes of vesicant-induced skin damage. The rat/human skin flap developed by Krueger et al. under contract DAMD17-82-C-2214 is an example of the potential use of the nude rat model.

Clinical evaluation and quantitation of cutaneous injury are still major obstacles in the study of vesicant toxicity, regardless of the animal model used. This problem currently is being addressed at our institute by the use of noninvasive measures of skin irritation and injury.

A paper on this subject by MAJ Westrom and an outline of vesicant research at LAIR appear in the Appendices B and C, respectively.

ANIMAL MODELS FOR VESICANT-INDUCED SKIN INJURY
Presented by MAJ Dale R. Westrom, MC

MAJ Westrom discussed the models currently available for use in the study of vesicants and the need for models that adequately reproduce the injury seen in human skin, particularly with respect to vesicles and bullae. He also pointed out that there is currently a lack of adequate measures of skin injury and urged the exploitation of noninvasive techniques.

- Disadvantages of animal models:

  1. Inability to form blisters
  2. Variability in response
  3. Difficulty in extrapolation of data to humans
  4. Expense and logistics
  5. Animal rights issues

- When choosing an animal model for cutaneous injury, several factors must be considered, including:

  1. The particular biological response to be studied
  2. The availability of the model

- The human skin is highly variable in structure and function. Skin thickness can vary from 2 to 10 mm. There are areas high in hair follicle density (scalp) and others relatively devoid of any skin appendages (lip).

- Pig skin is a good model. In comparisons of the relative skin thickness of several species, the pig integument approximates the thickness of the human. Density and size of hair follicles in pig skin are similar to human skin. In permeability studies, the porcine skin *in vitro* model demonstrated the suitability of pig skin. Furthermore, microvesicles with a split at the dermal-epidermal junction have been produced in pig skin using liquid neat butyl mustard (100 ug/cm^2).

- The athymic nude mouse:

  1. Will accept xenografts of skin and other tissues from a wide variety of donor species, including humans.

  2. Has been grafted successfully with both full- and split-thickness human skin (most of the studies in the literature have been with split-thickness human skin).

- Four types of skin are used for grafting at Letterman Army Institute of Research (LAIR):

  1. Abdominal

  2. Breast

  3. Facial

  4. Eyelid

- The grafted nude mouse:

  1. Graft survival is very dependent on skin thickness; therefore, only the relatively thin eyelid and foreskin are used for full-thickness grafts (only the full-thickness grafts have intact and viable skin appendages such as sweat glands and hair follicles).

  2. Success varies with the type of skin grafted, its thickness, and the group of mice being used at the time.

  3. Percent "take" is approximately 70% at LAIR.

  4. Grafts often survive for the life of the animal, i.e., from 0.5 to 1 year.

- Other immunodeficient animals, such as the cyclosporine-treated, athymic nude rat, have many of the same attributes as the athymic mouse. The nude rat has the advantages of being larger and hardier.

- The nude rat/human skin flap model is a somewhat complicated model that has human skin grafted onto the ventral aspect of an athymic nude rat. The grafted area and the blood vessels associated with it are then freed and made

into a pedicle flap. The pedicle is actually a sandwich of skin, with host skin on one side and grafted human skin on the other. The venous drainage from this flap can be directly accessed for measurement of skin metabolites and percutaneous penetration of compounds applied to the skin. (Editor's comment: Because nude rats are genetically immunocompetent, chronic cyclosporine is administered to prevent xenograft rejection.)

- The clinical evaluation and quantitation of cutaneous injury are still major obstacles to the study of vesicant toxicity.

- Noninvasive measures of skin function that are available or that soon will be available at LAIR include:

  1. Infrared thermography

  2. Laser doppler velocimetry

  3. Reflectance spectrophotometry

  4. Photopulse plethysmography

Summary and conclusions: There is currently no ideal model available; however, through the careful selection of specific animals for selected physiological responses, a large amount of valuable information on vesicant toxicity can be gained. There is also no good model for blistering, but the pig seems to have a number of advantages over other model systems. The grafted human skin/nude rodent models offer an unprecedented opportunity to study the metabolism and percutaneous penetration of highly toxic compounds on human skin. There is a definite need for better quantitative measures of skin injury.

A paper on this subject and an outline of vesicant research at LAIR appear as Appendices B and C, respectively.

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ARMY'S CURRENT RESEARCH PROGRAM IN MEDICAL COUNTERMEASURES TO VESICANTS

Presented by LTC Michael J. Reardon, VC
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LTC Reardon summarized some of the issues and established the groundwork for the subsequent sessions in which Workshop participants divided into groups to discuss the goals and objectives pertaining to vesicants. Key points made by LTC Reardon included the following:

- Programmatic issues of vesicant research:

  1. Vesicants as a threat have been overshadowed by organophosphorus compounds (OPs).

     a. There is an interest in increasing the emphasis and resources committed to medical countermeasures to vesicants.

     b. Large numbers of permanent civilian employees who are highly skilled and well trained currently work on OP-related projects. There is no intent to drop OP research and shift to vesicant work completely.

  2. The low priority of vesicant research has hindered the program.

     a. A technical base of some expertise has been maintained.

     b. At present, there is no program that can be depended on in the long-term to produce the needed fieldable end-items.

  3. Civilian research interest is limited.

  4. RAD V Program guidance calls for bringing the vesicant tech base effort up to approximately 10% of the medical chemical defense budget.

- Scientific and technical issues of vesicants:

  1. There is a rapid, irreversible action at the site of application.

  2. To be protective, decontamination must begin no later than 2 minutes after exposure.

  3. There is no animal model for formation of mustard blisters.

  4. Currently available Draize-type observations are unsatisfactory because they are neither quantitative nor objective. In addition, Draize testing has been viewed with some disfavor because of animal rights sensitivities.

  5. There are difficulties in the quantification of the mustard dose delivered to the skin.

  6. There are important issues relating to the relevance of research using simulants and analogs of surety agents.

- Current directions in vesicant research:

  1. Decontamination/detoxification

     a. There are currently no vesicant-specific candidates for decontamination/detoxification.

     b. An effort is currently under way to develop the Skin Decontamination Kit (SDK), part of an overall decontamination system, containing resin blends efficacious for vesicant decontamination.

  2. Topical barrier/protectants (It may be impractical to put a barrier on the human skin because it may affect normal functions; however, a topical barrier with decontamination/detoxification capabilities may still be of interest.)

     a. There is a current 6.1 effort, with no good candidates at the moment.

     b. The Canadians are proponents in the international arena of research in topical barriers.

3. Pretreatment

   a. This is a very attractive concept, but inherent problems include the question of how many different pretreatments a soldier can take before performance is adversely affected.

   b. There are no adequate model systems for making logical decisions for ranking candidate compounds.

4. Treatment

   a. Treatment has not been addressed as a separate issue.

   b. The lack of a blister model has been an impediment to the study of treatments for vesicant exposure.

- Current research:

1. Mechanism of action--mustard

   a. ICD and LAIR have ongoing investigations using cell and organ culture systems in addition to whole animal studies.

   b. There are approximately five contracts investigating some aspect of the pathogenesis of the mustard lesion.

2. Mechanism of action--lewisite

   a. Most of the research effort is being done at LAIR by Dr. McGowan.

   b. Vesicant research has had low priority, with lewisite at a lower priority than mustard.

3. Pretreatment compounds--mustard

   a. Several proposals have been reviewed, but most were not approved because of problems with scientific or programmatic merit.

   b. There are no accepted test systems available for evaluating compounds.

4. Pretreatment/treatment compounds--lewisite

   a. British anti-lewisite (BAL) is the most effective skin treatment.

   b. Although DMSA and DMPS are good systemic heavy metal chelators, there is no evidence of efficacy of either compound against dermal injury by lewisite.

TITLE: International Vesicant Research

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Although worldwide research efforts on vesicants have been intensified recently, particularly because of the use of mustard gas in the Iraq-Iran war, they still have failed to provide an adequate medical defense. Progress has been hampered by a lack of understanding of underlying biochemical and pathophysiologic mechanisms, the unavailability of valid and quantitative methods and appropriate animal models for assessing skin injury, and a lingering despair generated by decades of unsuccessful attempts at providing effective mustard antidotes. Most of the recent international efforts have been concentrated on developing preventive measures such as skin barriers, decontaminants, and chemicals that could detoxify mustards in vivo.

To provide "quick fixes," investigators have employed procedures (e.g., the Draize test) and skin models (e.g., rodent or pig skins) of doubtful validity to evaluate the efficacy of such preventive measures. The West Germans advocate the use of sodium thiosulfate and the Soviets promote the use of thiosulfate and Unithiol (2,3-dimercaptopropane-1-sulfonate, DMPS) for prophylaxis and/or early treatment of both skin and systemic exposures to HD. At best, the efficacies of these compounds have not been fully established; at worst, they have been found to be either ineffective or even detrimental in vivo.

Research needs not presently addressed include criteria for the rigorous diagnosis and prognosis of exposures to HD, procedures that are critical for effective triage and for optimal treatment of HD casualties. Studies on the development of new quantitative, highly sensitive, and specific procedures for the detection of HD in human blood cells and tissue have just begun.

Because of the extensive use of mustards and related alkylating agents in the treatment of hyperplastic and neoplastic diseases, it is not surprising that some of the most significant recent contributions have come from cancer research. Whereas research on the killing of tumor cells and tissues by antineoplastic agents provides significant information on toxic mechanisms, studies on the prevention and treatment of unwanted side effects furnish new and potentially useful antidotal approaches against HD-induced injury. The scientific literature is also replete with descriptions of improved and innovative procedures for evaluating skin injury that could prove useful in future HD research. Several other papers presented at this conference contain valuable information relating to recent contributions by the United States on the mechanism of cutaneous HD injury, skin models used for assessing its severity, and the development of a new generation of skin decontaminants/detoxicants.

This presentation will focus on only a few examples that were selected from the literature because of their relevance to the mustard problem: (a) the c x t (concentration x time) concept for defining a given cutaneous HD dose; (b) the dose-response relationships between the HD dose and the fixation of HD in skin, the doses of HD that are effective for treating psoriasis, and the doses of HD that result in increasing severities of skin injury; (c) the analysis of suction blisters as a potential in vivo assay for skin injury; (d) the analysis of enzyme leakage from epidermal slices as a potential in vitro assay for skin injury; and (e) the types of manipulations of the glutathione system that are possible and that could lead to new pretreatment approaches.

The examples cited are not intended to exclude other approaches but were selected to illustrate the kind of information that could be exploited now to establish short- and intermediate-term, state-of-the-art HD research programs. The long-term effort could be directed to maintaining and enlarging in-house and contractual expertise and promoting a state of creative vigilance under which optimal use of emerging technologies and concepts can flourish.

INTERNATIONAL VESICANT RESEARCH

Presented by Bruno Papirmeister, Sc.D.

Although world military research efforts on medical defense against sulfur mustard have intensified recently--largely because of the use of this vesicant in the Iraq-Iran conflict--no significant advances have been reported to date. While our knowledge of pathophysiology of the mustard injury has been significantly advanced in the United States, further validation is needed and exploitation of the proposed mechanism for therapeutic purposes has been hampered by the unavailability of valid test procedures and appropriate animal models. Most countries continue to advocate the symptomatic management of the cutaneous, respiratory, eye, and systemic effects of mustard exposure and stress the importance of preventive measures such as contamination avoidance, decontamination, and detoxification. The Federal Republic of Germany and USSR are stressing detoxification of mustard *in vivo* and have included sodium thiosulfate and Unithiol (2,3-dimercaptopropane-1-sulfonate, DMPS) for the pretreatment and/or early treatment of both skin and systemic mustard exposures. However, recent studies with these compounds failed to demonstrate any efficacy for attenuating the cutaneous mustard injury.

Research needs have been identified as follows: (a) establishment of criteria and methods for a rigorous, early diagnosis and prognosis of mustard exposures, procedures that are critical for meaningful triage and for optimal treatment of casualties; (b) development of valid *in vivo* and *in vitro* methods and models for dosing and for quantitative assessment of mustard injuries to the skin, eyes, respiratory tract, and other body systems and organs; (c) development of improved skin barriers, detoxicants, and decontaminants coupled with simple and quantitative methods for assessing their efficacy; (d) development of effective pretreatment drugs and procedures and methods for evaluating their efficacy; and (e) improved medical management of mustard casualties and promotion of the healing process. Examples from the scientific literature were presented to illustrate some potentially fruitful approaches.

- Cutaneous mustard dosing: F. Schwartz (1937) dissolved mustard in vaseline and used the c x t concept (concentration in percent x time in seconds) for establishing reproducible erythematogenic, vesicant, and necrotizing doses in humans (Table 1).

- Therapeutically effective cutaneous mustard dose: L. Illig et al. (1979) used mustard-vaseline ointment to treat psoriasis and mycosis fungoides patients. Using the c x t concept (Table 1) and the information reported by Illig for the distribution of HD in human skin (Table 2), the therapeutically effective dose was calculated to be about 1/50 of the vesicating dose.

- Skin injury assessment in vivo (a possible replacement for the skin Draize test): M.C. Middleton (1981) used a suction blister technique to obtain good dose-response data with a skin injurant agent. He analyzed the suction blister fluid for the presence of cytoplasmic, mitochondrial, and lysomal enzymes (Figure 1).

- Skin injury assessment in vitro: M.C. Middleton (1981) obtained good dose-response data by measuring enzyme leakage into the culture medium following exposure of epidermal slices to a skin injurant agent (Figure 2).

- Pretreatment with radioprotective thiols: Walker and Smith (1969) found that dithiols (e.g., dithiothreitol) that penetrate the cell membrane provide protection against mustard cytotoxicity (Figure 3; Table 3).

- Pretreatment possibilities against mustards by boosting the body's own detoxification capability: The glutathione system--the major natural detoxification system for mustards--can be made more effective by inducing glutathione transferase and/or by providing glutathione esters capable of penetrating all membranes. Such pretreatment might be effective against cutaneous, eye, lung, and systemic injuries (Figures 4-7; Table 4).

- Healing of the cutaneous mustard injury: Recent reports show that several growth factors accelerate the healing of thermal skin injuries. If similar positive results can be achieved for cutaneous mustard injuries, the lengthy convalescence of mustard casualties might be significantly shortened.

Table 1. The c x t Concept for Cutaneous HD Exposures in Humans (Adapted from Schwartz 1937)

Severity of Skin Injury	Dose of HD (c x t)[a]
Erythema	300[b]-1500
Vesication	1500-3000
Necrosis	>3000

[a]The concentration (c) of HD is given in percent; that is, pure HD is designated as 100, 5% salve in vaseline as 5, etc. The time (t) is in seconds.

[b]If a strong reaction results at a c x t = 300, the individual is considered to be highly sensitive to HD.

Table 2. Distribution of Radioactivity in Human Skin 3 Hours after Application of Salve Containing Radiolabeled HD[a] (Adapted from Illig et al. 1979)

Patient No.	Radioactivity (pCi/mg fresh tissue) in: Unaltered skin	Psoriatic skin
1	I = 7.96	I = 13.1
	II = 0.90	II = 0.44
	III = 0.22	III = 0.77
2	I = 3.0	I = 3.44
	II = 1.36	II = 1.24
	III = 0.70	III = 0.52
3	I = 3.52	I = 4.13
	II = 1.56	II = 1.05
	III = 0.50	III = 0.52

[a]Salve used for treatment of psoriasis contained 0.005% HD (U-^{14}C; specific activity, 10 Ci/mole) in vaseline (so-called Russian Ointment). Punch-biopsy materials were obtained 3 hours after application of 0.25 g of HD salve to 100 cm^2 of skin of psoriatic patients.

[b] I = Epidermis plus upper dermis
II = Middle plus lower dermis
III = Subcutaneous fatty tissue

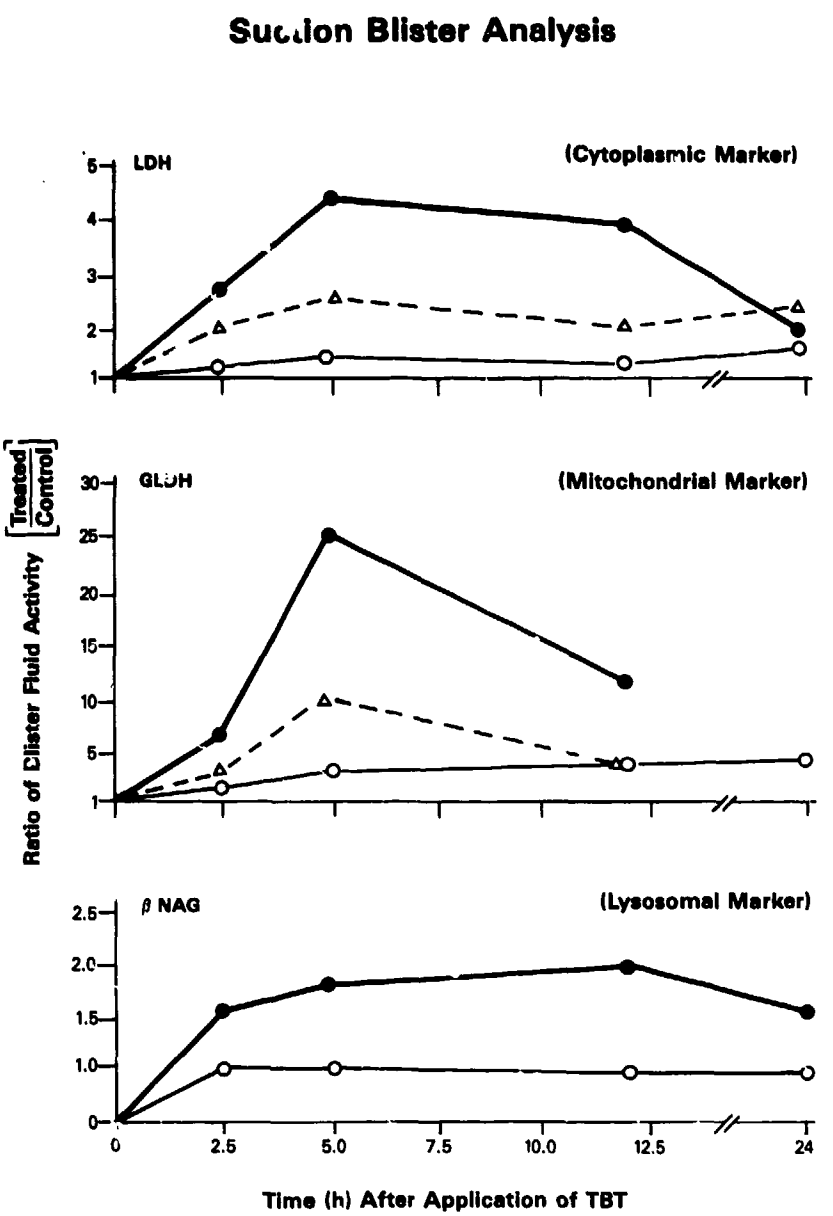

Figure 1. Enzyme activities in suction blister fluid from rat dorsal flank skin treated with the dermatotoxic chemical tributyltin (TBT) were compared with those from solvent-treated flanks of the same animals. Cutaneous applications were as follows: TBT at 0.3 umol/cm^2 (solid circles) or 0.15 umol/cm^2 (triangles), or plasma from animals treated cutaneously with TBT at 0.3 umol/cm^2 (open circles). Values are means for five animals. LDH, lactate dehydrogenase; GLDH, glutamate dehydrogenase; and βNAG, N-acetyl-β-glucosaminidase. (Adapted from Middleton 1981)

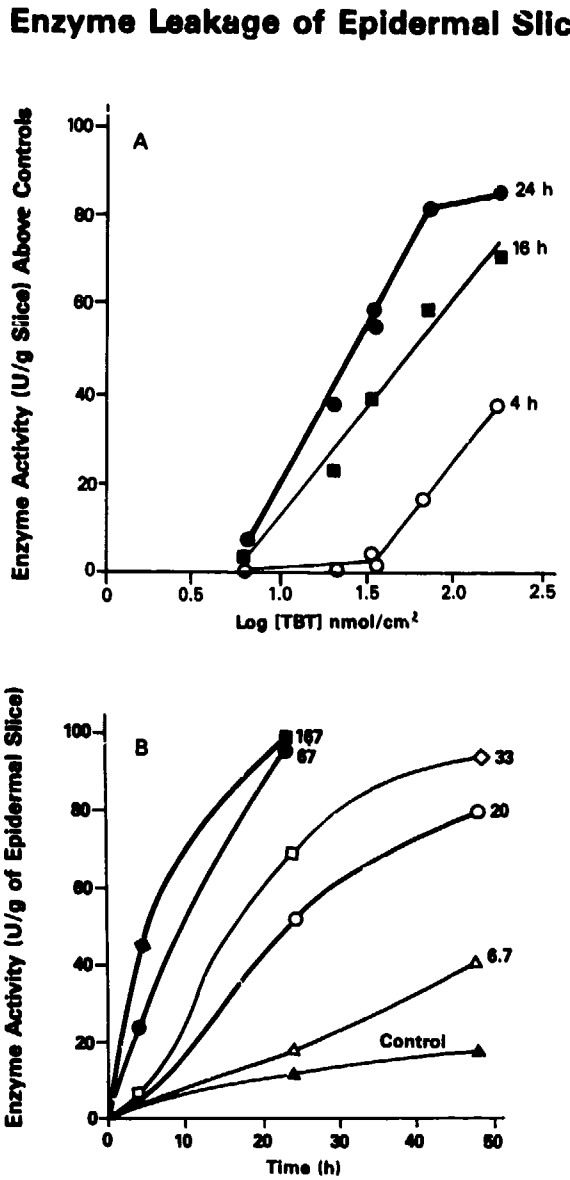

Figure 2. Dorsal skin of 4-week-old male rats was treated with tributyltin solution and excised 15 minutes later. Epidermal slices were floated epidermal side up in medium. Malate dehydrogenase activity in the medium was measured at the indicated times. Tributyltin doses are given for curves in (B) as nmol/cm^2. (Adapted from Middleton 1981)

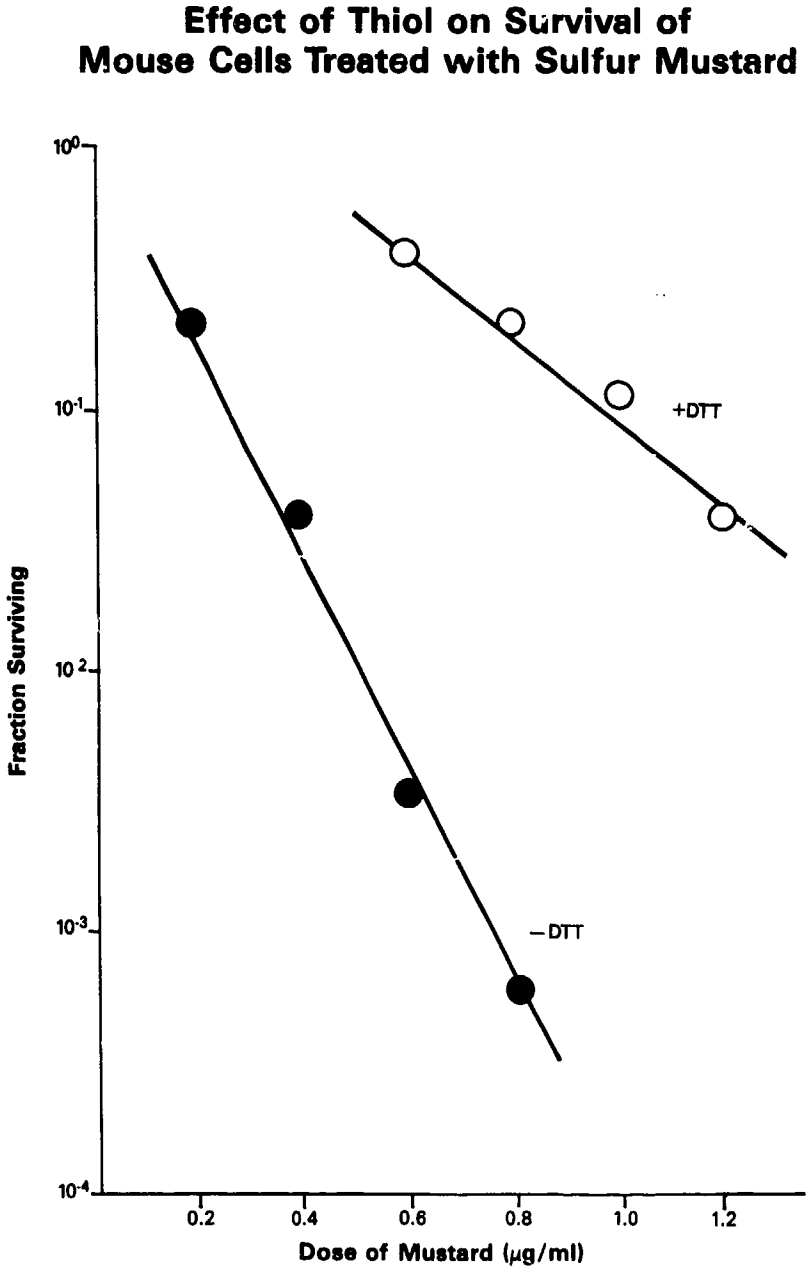

Figure 3. Mustard dose-survival curves for L-strain mouse cells were determined in the presence and absence of 10^{-3}M dithiothreitol. Cell survival was measured as plaque formation in petri dish cultures. (Adapted from Walker and Smith 1969)

Table 3. Effect of the Thiol Dithiothreitol (DTT) on Alkylation of Macromolecules by Sulfur Mustard (Adapted from Walker and Smith 1969)

Dose of mustard (ug/ml)	Extent of alkylation (d.p.m./ug)					
	DNA		Protein		RNA	
	-DTT	+DTT	-DTT	+DTT	-DTT	+DTT
0.2	15.8	----	2.52	----	----	----
0.4	31.8	----	2.19	----	----	----
0.6	62.1	35.4	5.43	2.58	----	----
0.8	52.7	39.0	6.40	4.21	----	----
1.0	94.0	52.8	6.74	3.50	30.7	14.5
1.0	114.0	34.5	8.44	4.12	36.4	18.7
1.0	----	53.4	----	4.16	----	----
1.2	----	62.5	----	6.74	----	----
$\frac{y/x}{N}$[a]	89.3	50.1	8.39	4.49	33.6	16.6
Alkylation reduction factor	1.8		1.9		2.0	

Mouse L-cell suspension cultures were treated with ^{35}S-labeled mustard for 1 hour in the presence or absence of 10^{-3} M DTT.

[a] y is the extent of alkylation at dose x.

Manipulations of the Glutathione System

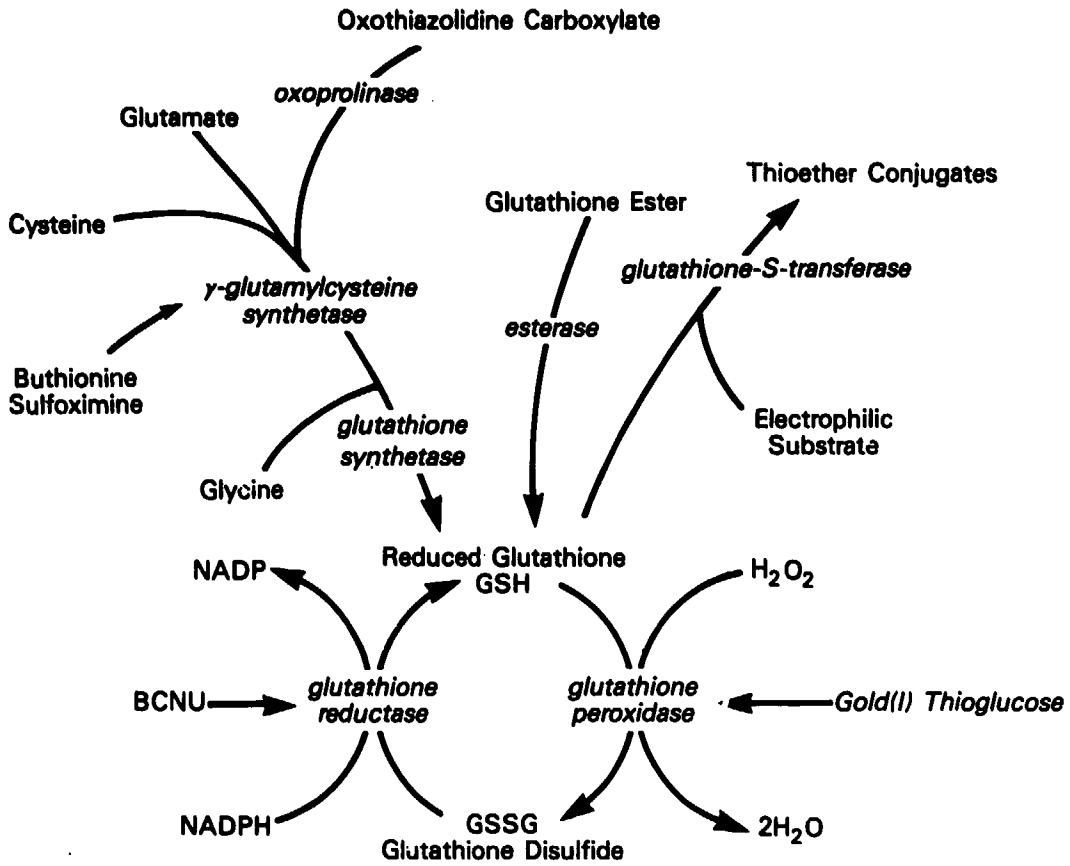

Figure 4. Reactions that result in glutathione synthesis and depletion are shown, along with inhibitors of three relevant enzymes. Buthionine sulfoximine selectively inhibits an enzyme in glutathione biosynthesis. 1,3-Bis(chloroethyl)-1-nitrosourea (BCNU) inhibits glutathione reductase, which regulates the ratio of reduced to oxidized glutathione. Gold(I) thioglucose inhibits glutathione peroxidase, which mediates detoxification of peroxidase. Not shown are other conditions that affect glutathione concentration and oxidized/reduced glutathione ratios. (Adapted from Clark 1986)

Potentiation of Cytotoxicity by an Inhibitor of Glutathione Synthesis

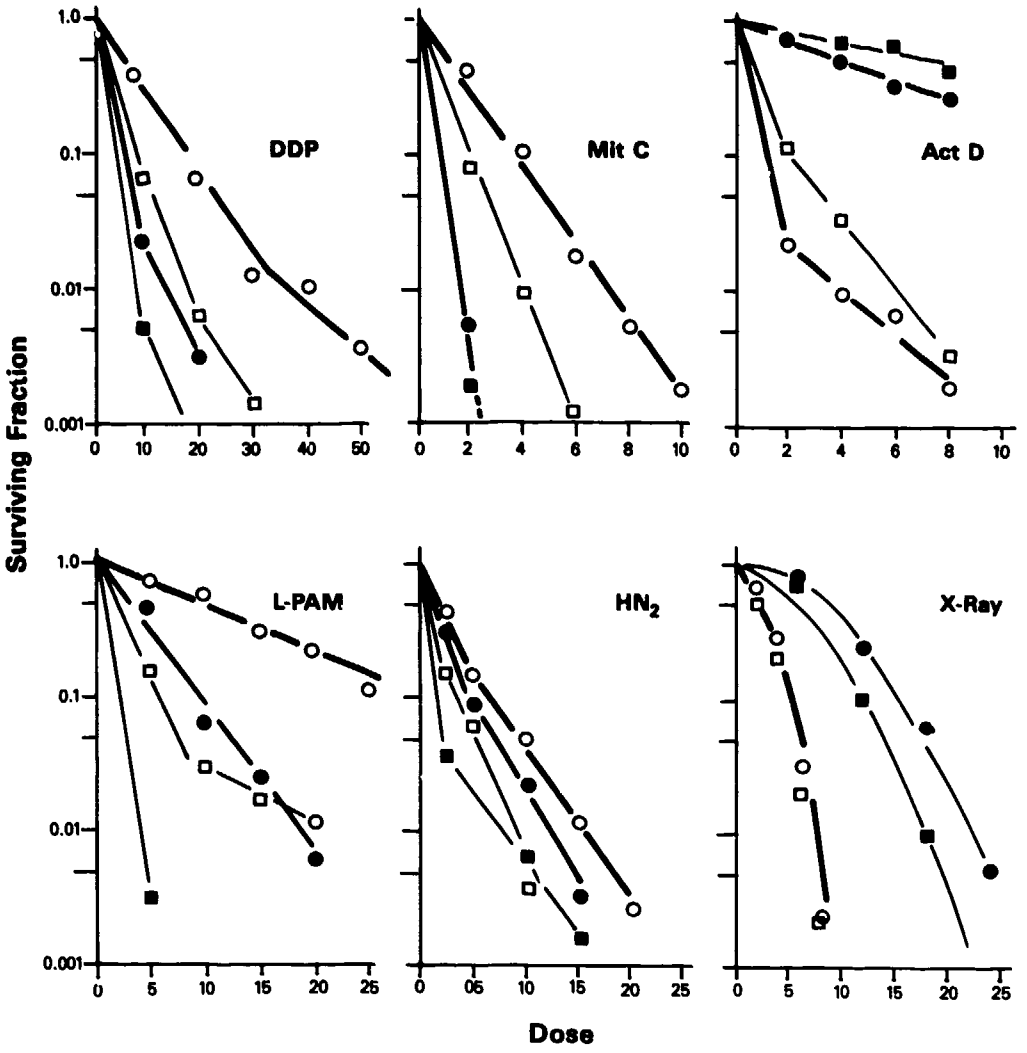

Figure 5. Cultured EMT6/SF mouse tumor cells were treated for 12-14 hours with 50 uM buthionine sulfoximine (BSO), an inhibitor of glutathione synthesis (squares). Control cells were not treated with BSO (circles). Cells were then exposed to drug or X-irradiation under aerated (open symbols) or hypoxic conditions (solid symbols). Dose units: uM for chemotherapy agents; grays for X-rays. Drugs: DDP, cis-dichlorodiammino Pt(II); MitC, mitomycin C; ActD, actinomycin D; L-PAM, L-phenylalanine mustard; and HN_2, nitrogen mustard. (Adapted from Shrieve and Harris 1986)

Glutathione Esters Protect Cells Against Irradiation

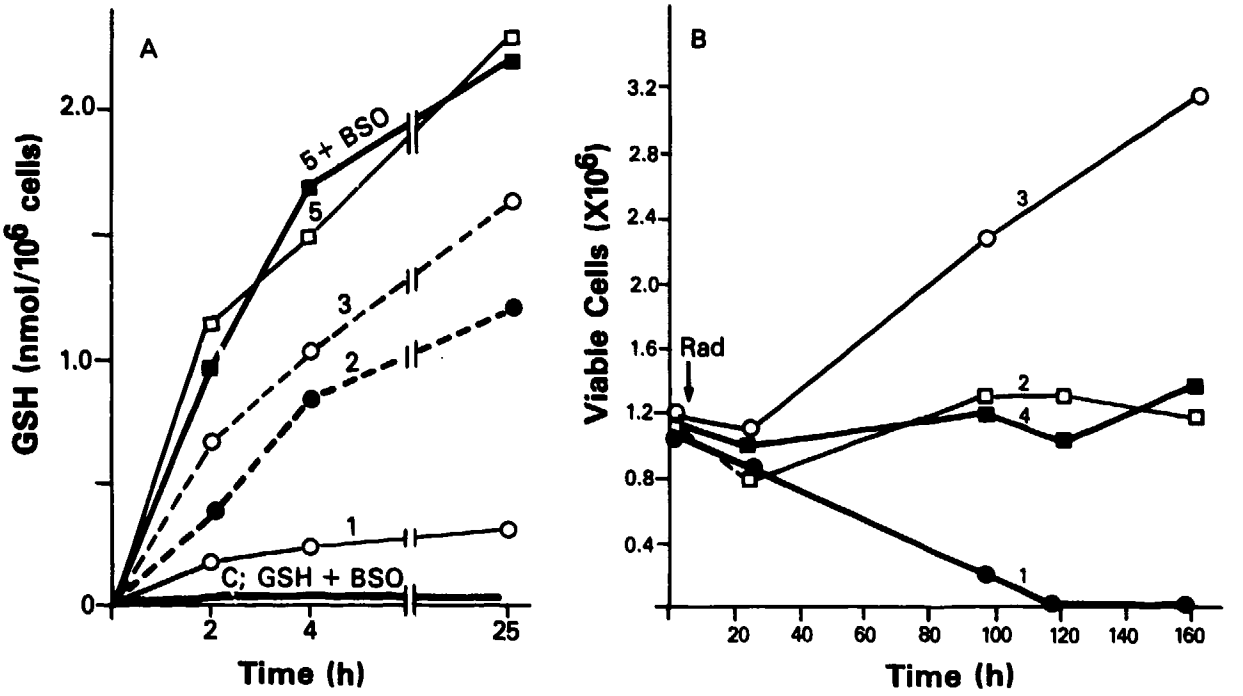

Figure 6. Human CEM lymphoid cells were depleted of glutathione by incubation with buthionine sulfoximine (BSO) before use in all experiments. (A) Effect of concentration of glutathione monoethyl ester (GSH ester) in medium on cellular glutathione levels. Numbers on curves indicate GSH ester concentrations in mM. The bottom curve represents two experiments: the control (C), with no additions; and an incubation with 5 mM glutathione plus 1 mM BSO. (B) Protection of CEM lymphoid cells against radiation damage by GSH ester. Cells were irradiated (500 rads) at 3 hours. Additions to incubations were as follows: 1, none (control); 2, GSH ester after irradiation; 3, GSH ester before irradiation; and 4, glutathione before irradiation. (Adapted from Wellner et al. 1984)

Induction of Glutathione S-Transferase Enhances Detoxification of Aflatoxin

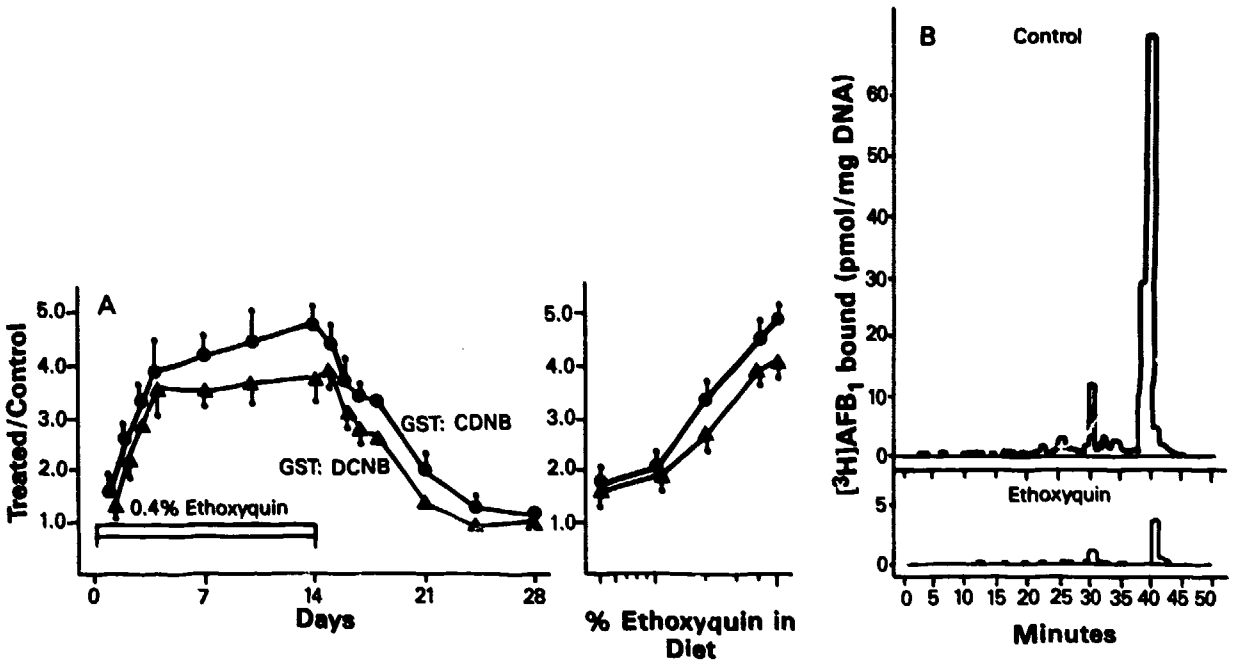

Figure 7. (A) Glutathione S-transferase (GST) was induced in rats by ethoxyquin as dietary supplement. The ratio of hepatic GST activity in ethoxyquin-treated rats to that in control rats is shown for two GST substrates, 1-chloro-2,4-dinitrobenzene (CDNB) and 3,4-dichloronitrobenzene (DCNB). The time course of GST induction (left) and the dose dependence of the effect (right) are shown. (B) High pressure liquid chromatography profiles are shown for aflatoxin B_1 (AFB_1) adducts of rat liver DNA. Rats fed control or ethoxyquin-supplemented diets were gavaged with [^{3}H]AFB_1 (250 ug/kg), and sacrificed for DNA isolation 2 hours later.

Table 4. Effects of Ethoxyquin-Supplemented Diets on Toxicity of Aflatoxin B_1: Bilary Elimination of Aflatoxin-Glutathione Conjugates (AFB_1-SG) and Formation of Hepatic Foci (Adapted from Kensler et al. 1986)

	Dietary treatment	
Parameter	Control	Ethoxyquin 0.4%
Bilary elimination of AFB_1-SG in 2 hours		
AFB_1-SG (nmol)	0.65±0.12[a]	2.71±0.47
(% of AFB_1 dose)	1.1	5.1
γ-Glutamyl transpeptidase-positive foci in liver		
Incidence at 16 wk	12/12	3/10
Foci (No./cm^2)	1.60±0.28	0.04±0.02[b]
Volume of foci (% of total liver)	0.11±0.026	0.0003±0.0001[b]

In the bilary elimination study, rats were fed the ethoxyquin or control diet for 1 week, then administered a single oral dose of AFB_1 (250 ug/kg). Bile was collected via cannula for a 2-hour sampling period and analyzed by reverse-phase liquid chromatography. In the hepatic pathology study, rats received two doses of AFB_1 and were sacrificed at 4 months.

[a]Data are means±S.E.; five animals/group in bilary study; numbers as given in incidence data in hepatic foci study.

[b]$P<.01$ relative to control.

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DISCUSSION

Session IV

It was suggested that measuring the amount of radiolabeled mustard that fixes to the skin would be sufficient for screening decontaminants and topical barriers, since the amount that fixes to the skin determines the severity of injury. In response, it was pointed out that measurement of radiolabeled mustard affixed to the skin may not discriminate between active and inactivated mustard, since the test compounds may function by inactivating the mustard without affecting its ability to affix to the skin.

Systemic effects of mustard were not included in the list of current research. In response to a query about whether this was intentional or an oversight, the omission was described as not intentional. Ultimately, all aspects of injury would be investigated.

COL Denniston pointed out that the presentation on international research by Dr. Papirmeister did not address ongoing research, but instead discussed application of techniques. In response, the omission was described as an indication of the paucity of current international research.

There was a discussion to clarify whether the dust problem associated with use of the SDK, as described by LTC Harrington, would create a problem with off-gassing of toxic substances. It was emphasized that there is no charcoal in the SDK, and that the resins are loaded onto a fiberlike material. After the user tests were performed, a prototype packet in which the resins are packaged in a "powder puff" of cheesecloth was designed to counter the problems of dusting. Other tests have shown that there is less off-gassing with the resins than with Fuller's earth.

The importance of U.S. leadership in mustard research was emphasized; a reduction in this research effort would have a detrimental effect on our international commitments (e.g., NATO). It was noted that our dollars are driven by the Deputy Chief of Staff for Research and Development for Acquisitions and triservice requirements and that, at this time, there is limited interest in doing research for developing medical countermeasures to vesicants.

Session V

New Directions

NEW DIRECTIONS FOR THE VESICANT DEFENSE PROGRAM

The consolidated statement of Groups A and B covered two principal subjects: deficiencies identified in the mission area analysis; and research program goals, objectives, and implementing tasks.

1. Programmatic Research Deficiencies

The working groups were asked to address current deficiencies identified in the mission area analysis from the various viewpoints represented at the meeting. These deficiencies were described as major conceptual gaps in data, material, research programs, resources, or procedure/doctrine which need to be filled to define and develop a responsive tech base program.

The working groups identified the following deficiencies impacting valid, timely, and effective program execution:

a. Lack of a formalized definition of the problem in terms of user community needs and likely exposure scenarios; this is essential for rational development of research plans

b. Lack of medical management items or procedures in the overall development pipeline

c. Lack of a clear set of goals and objectives for the vesicant area from which to develop a research program prioritized with respect to needs and resources

d. Lack of critical tools with which to implement a clearly focused research program (e.g., animal models, standardized challenge exposures for vesicant agents, neat agent facilities, validated simulants, and accepted criteria of efficacy for pretreatment and treatment compounds)

e. The tech base, which contains many valid but older studies, is not consolidated, and is neither readily available to nor even known by many current researchers and research planners

f. Failure to adequately communicate DoD vesicant defense research needs to the scientific and academic communities (many qualified scientists probably do not receive copies of the Broad Agency Announcement [BAA] or the Commerce Business Daily [CBD])

g. Editor's comment: Failure to specifically identify the need for a topical protectant/barrier against vesicants and other chemical warfare agents in relevant DoD documents, e.g., JSA Requirements and BAA

2. Research Program Goals, Objectives, and Implementing Tasks

The working groups developed sets of goals and objectives to remedy deficiencies in mission area analysis, along with tasks to implement these goals and objectives. Since there is an obvious correlation between an objective and the tasks which address it, they are presented together. Tasks are listed under the objective they support.

The various formulations of the purpose of the USAMRICD vesicant program can be distilled to a single goal: to develop medical countermeasures and procedures to preserve the fighting force.

To achieve this goal, the following objectives and implementing tasks were identified:

Objective 1: Clearly define the vesicant threat.

a. Conduct studies to predict probable exposures under field conditions, considering: (i) level of protection available; (ii) type of agent (HD, L, HL, etc.); (iii) form of agent (liquid, aerosol, vapor, thickened, dust); (iv) probable range of doses; and (v) probable numbers and types of casualties.

b. Develop and validate models to predict impact of probable exposures on force effectiveness: (i) expected numbers and types of injuries (ocular, respiratory, skin, systemic, mixed agent/conventional casualties); and (ii) impact of expected injuries on task and mission performance.

c. Use models from Task b to evaluate effect of current and potential medical countermeasures on force effectiveness/ mission performance.

d. Develop prioritized "needs" list of countermeasures with the most positive impact on force effectiveness. (Editor's comment: Include in this list topical protectants/ barriers as a specific need in the JSA Requirements and BAA.)

Objective 2: Develop and consolidate tech base information in a form that can be used to support Objectives 3 and 4 (presented below).

a. Collect and centralize documents in a format that can be accessed easily and rapidly.

b. Categorize, catalog, and cross-reference types of available data.

c. Identify data supporting quick fixes and addressing current issues and research plans.

Objective 3: Improve the current state of medical management information on vesicant exposure.

a. Consider quick-fixes: (i) refielding BAL ointment and BAL in oil for lewisite exposures; (ii) supporting update of field manuals to ensure accuracy and consistency of information; and (iii) evaluating barrier and decontaminant potential of already fielded materials.

b. Institute procedures for collection of clinical data on future human casualties of vesicant exposure: (i) develop and formalize data to be collected; (ii) identify resources needed (personnel, equipment, budget); and (iii) use data to support medical management (Objective 3) and research plans (Objective 4).

c. Develop a functional triage system: (i) prepare criteria for accurate assessment of exposure consequences for evacuation and for return to duty (output of Objective 4, Task c); (ii) develop resource cost vs. benefit tables for evacuation; (iii) predict time to incapacitation for types of injuries to provide options to commanders for different combat scenarios; and (iv) periodically update the triage system based on results of research programs (Objective 4).

d. Train medical personnel in treatment of vesicant injuries. (Editor's suggestion: One approach would be to increase the number of health care providers attending the Medical Management of Chemical Casualties Course and increase the emphasis on vesicants in the course.)

e. Develop individual/casualty decontamination strategies compatible with field operations and/or other medical problems: (i) define minimal adverse impact on force effectiveness (modeled in Objective 1, Task c); (ii) determine minimal requirements for water and manpower; (iii) improve ability to detect contamination; (iv) define contamination thresholds (from Objective 4, Task c); and (v) improve the anti-vesicant efficacy of the skin decontamination kit (in advanced development).

f. Support development of new medical management doctrine and procedures to integrate the products of Objective 4 into field medical systems.

Objective 4: Develop, implement, and periodically update short-term (0- to 2-year), medium-term (2- to 5-year), long-term (5- to 10-year), and strategic (>10-year) research plans to address needs identified in Objectives 1 through 3 with the resources available.

a. Support development of quick fixes (e.g., BAL refielding and/or reformulation).

b. Address deficiencies in available research tools: (i) whole animal models, if needed; (ii) organ system models for predicting injuries (priority of organ systems determined in Objective 1, Task b); (iii) in vitro models to facilitate compound screening; (iv) dosimetry--standardize; and (v) vesicant agent simulants--validate for specific research uses.

c. Support development of criteria for: (i) advancing compounds (barriers, pretreatments, prophylaxis, treatments, decontaminants) through development and fielding; (ii) accurate assessment of consequences of exposure (support improvement of triage system in Objective 3, Task c); (iii) return to duty (support to Objective 3, Task c); and (iv) contamination and level of decontamination needed (support to Objective 3, Task e; often stated as "How clean is clean?").

d. Develop research approaches to address: (i) prioritized end-product needs; (ii) criteria development (see Task c); and (iii) research tools (see Task b).

e. Estimate resource requirements of the research approaches proposed in Task d and identify available intra- and extramural resources: (i) personnel; (ii) facilities, to include neat agent facilities; (iii) budgets; and (iv) time to completion.

f. Evaluate payoff of research approaches proposed in Task d in terms of preserving force effectiveness (see Objective 1, Task c).

g. Sort the achievable from the desirable: (i) scientific feasibility and probability of success in an acceptable time frame; (ii) payoff to force effectiveness; and (iii) resource availability, to include solicitation of extramural resources.

Objective 5: Integrate the medical vesicant defense research program and medical management programs with existing and emerging quad-service doctrine, plans, training, and operations.

a. Improve interservice communication through: (i) a directory of offices; and (ii) a central meeting calendar and clearinghouse for information.

b. Promote understanding of existing systems for joint service cooperation.

c. Consolidate/coordinate systems, requirements, and reporting processes for joint service cooperation.

d. Ensure consistency of service education and training.

Objective 6: Improve communication of vesicant defense needs to the scientific and academic communities.

a. Expand the distribution of the BAA to include universities.

b. Advertise the availability of funding for relevant DoD research via the CBD and BAA in appropriate scientific, medical, and veterinary journals.

WORKSHOP ASSESSMENT

A. Introduction

The Vesicant Workshop held by USAMRICD at The Johns Hopkins Kossiakoff Center, 3-5 February 1987, was highly successful. It was unusual in its approach (an in-depth, up-front analysis on a difficult research area) and its composition (Joint Services representatives of both the Combat and Materiel Developers). Recent use of vesicants by Middle East combatants has underlined our need to counter the vesicant threat, and USAMRDC plans to allocate increased resources for medical countermeasures and medical management items. The objective of the Workshop was to help focus these increased efforts by providing the basis for developing a realistic research plan. The steps envisioned to produce this plan include:

1. Identifying critical user needs on the basis of maximized force effectiveness

2. Identifying technologic requirements and capabilities

3. Estimating the cost and time required for proposed solutions to these needs

4. Matching available resources against estimated costs per solution (facilities, time, people, expertise)

5. Sorting the achievable from the desirable based on step 4

6. Prioritizing the achievable on the basis of user needs and combat developer requirements

7. Identifying and tasking the appropriate DoD agency to address each need and requirement.

The Workshop participants included the Army Combat Developers (Chemical School, Academy of Health Sciences), representatives from three military services, and officers from the medical chemical defense research program. The Workshop consisted of one and one-half days of background briefings and one day of working group sessions during which two groups (half of the attendees each) addressed the same questions and issues. Group A consisted primarily of participants

from the tri-service user community and combat development agencies. Group B consisted primarily of participants from research program areas. The working groups addressed:

1. Present mission area deficiencies

2. Program goals and objectives

3. Tasks required to address goals and objectives

During the Workshop, the scientific community presented the status of the tech base and highlighted promising research approaches and significant deficiencies. The user community emphasized their need to maximize force effectiveness and encouraged the inclusion of this concept into cost/benefit analyses. It became clear that the scenarios of vesicant exposure that are most likely to result from present fighting doctrine are not well established, nor are the relative benefits to force effectiveness of addressing eye versus skin versus respiratory versus systemic exposures quantitatively understood. This Workshop opened lines of communication between users, researchers, and materiel developers. Continued communication and further front-end analysis will be essential to the development of a cogent USAMRICD research plan.

The following section presents a synopsis of key findings distilled from the three days of presentations, discussions, and working groups.

B. Key Findings

1. Combat developer input and dialogue with the materiel developer early in the research planning cycle is important to focus efforts and optimize use of limited resources. This helps the users to define and prioritize their needs because they know what is feasible and practical, and it helps the researchers to target their efforts and resources because they know what is needed. Furthermore, coordination of the tri-service users, combat developers, and materiel development communities should be an ongoing process.

2. Although a large tech base already exists that includes analysis of the vesicant experiences of World War I and subsequent research, these data have not been fully accessed or analyzed for use by current researchers. The tech base is not adequate to solve today's problems, but it does contain important data, much of which could not be reproduced today because of legal and human rights considerations. The available data should be accessed and synopsized to establish our baseline knowledge.

3. Vesicant program priorities and resources have fluctuated over the years. Real progress will require relatively stable resources and a stable core group of workers for the duration of the research plan.

4. Realistic time lines for research and development need to be set and coordinated with the Army combat development agencies. Every project cannot be completed in 5 years to accommodate budgeting schedule cycles.

5. We should apply lessons learned from starting up the organophosphorus agent tech base program. For example, concentrating on ameliorating effects on one organ system generally resulted only in unmasking toxic and incapacitating effects on other organ systems. It is recommended that vesicants be approached with cognizance of the effects of agent and countermeasures on the whole organism, and the effects on all target organs should be considered.

6. The majority of past vesicant research efforts focused on skin lesions, their mechanisms, and treatment. It is not clear that protecting this agent target (skin) is the most important for maximizing force effectiveness. Although some personnel may function with skin blisters in noncritical areas, they would be incapacitated by eye or respiratory exposures of comparable dose. The likely exposure scenarios (level of available protection, form and type of agent, and probable dose ranges) would determine projections of injuries and their sequelae. The relative importance of these sequelae to force effectiveness needs to be analyzed in concert with the user community.

7. Vesicant agents produce irreversible damage within the first 1-2 minutes after exposure, even though symptoms of that damage may not appear for many hours in the case of mustard exposures. This seriously complicates diagnosis, triage, and the development of effective strategies for treatment of vesicant injuries.

8. There are questions about whether and how vesicant-induced lesions should be managed differently from thermal burns. The data on vesicant lesion healing rates should be examined in order to partially address this question (see para. 2).

9. The inadequacy of *in vivo* and *in vitro* models hampers vesicant research. There were some suggestions for possible animal skin models, including discrete areas of animal skin that vesicate during disease states (e.g., pox blisters on the underbelly of pigs). However, there is little agreement on the most promising directions for developing other organ system models or simple *in vitro* screening techniques.

10. The existing knowledge of vesicant effects does not support the development of rationally based criteria for screening pretreatments, antidotes, and therapies for vesicant injuries. At the present time, screening would have to be empirical and based on arbitrarily chosen model systems. There is general agreement that the criteria that eventually will be developed must be based on efficacy against incapacitating injuries rather than against lethality, but the most appropriate models for this criterion and the quantitative level of efficacy that would constitute success are unknown.

11. The utility of medical information emanating from the recent use of vesicants in the Iran-Iraq conflict has been limited by a paucity of systematically collected clinical data. As part of the Workshop, a working group was formed to identify clinical questions to be addressed, tests to be performed on casualties, and personnel and equipment required to obtain useful information in the future.

12. Current U.S. military medical field manuals contain information on the medical management of vesicant injuries which may be inconsistent with current knowledge.

13. One quick fix needed is to develop a system which allows commanders and field medics to predict the nature, severity, and time course of the medical sequelae for vesicant exposures in order to determine whether further fighting is possible for an exposed person or whether that person should enter into the casualty-handling system. This is presently seen as a functional triage system to maximize force effectiveness. Options available to commanders and medical triage personnel include continuance of battlefield service following some medical attention and the early evacuation of an exposed but presently functional person before he becomes incapacitated.

14. Another quick fix that will be considered is the refielding of BAL in oil and/or BAL ointment for treatment of lewisite exposures.

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Appendices

APPENDIX A

TREATMENT OF PATIENTS WITH CUTANEOUS VESICANT INJURY

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I. Burns as war wounds

Conflict	Burns as a percentage of all casualties
RVN 1965-1973	4.6
Falkland Islands 1982	18.0
Yom Kippur War 1973	10.5
Lebanon 1982	8.6

II. Systemic effects of burn injury: Proportional to extent of burn

Organ system	Early response	Later response
Cardiovascular	Hypodynamic	Hyperdynamic
Pulmonary	Hypoventilation	Hyperventilation
Endocrine	Catabolism	Anabolism
Central nervous system	Agitation	Obtundation
Urinary	Oliguria	Diuresis
Gastrointestinal	Ileus	Hypermotility
Skin	Hypoperfusion	Hyperperfusion
Immune	Hyperreactive	Hyporeactive

III. Resuscitation

A. Maintain adequate airway

B. Establish secure intravenous line using large-caliber cannula

C. Administer crystalloid fluids

D. Place urethral catheter and monitor hourly urinary output

E. Maintain peripheral circulation

F. Decompress upper gastrointestinal tract

G. Treat wounds (early priority in chemical burns)

IV. Vesicant

A. Surety agents

1. Sulfur mustard (H)

2. Lewisite (L)

3. Phosgene oxime (CX)

B. Tissue effects

1. Local

a. Skin injury--inflammation, blistering, ulceration

b. Mucous membranes--inflammation and cell death

c. Inhalation injury--inflammation and cytotoxicity

d. Upper gastrointestinal tract--inflammation and cytotoxicity

2. Systemic
    a. Malaise, vomiting, fever
    b. Hematologic effects
    c. Small bowel mucosal necrosis
    d. Mutagenic action and respiratory cancers

C. Determinants of injury severity
    1. Extent of surface exposed
    2. Concentration of agent
    3. Duration of contact
    4. Tissues exposed

D. Initial treatment
    1. Immediate and copious water lavage
    2. Irrigation of eyes
    3. Removal of all contaminated clothing
    4. Fluid resuscitation
    5. Evaluation of airway
        a. Endoscopic examination
        b. Tracheal intubation and mechanical ventilation, if indicated
    6. Assessment of esophageal injury, if indicated

E. Later care
    1. Debridement of vesicles during wound cleansing procedure
    2. Topical burn wound chemotherapy
    3. Monitoring of pulmonary function
    4. Fluid and nutritional support

5. Infection surveillance

6. Long-term cancer surveillance

F. Burn patient triage

1. Intensity of care related to extent of burn

1-20% - Minor injury, can delay hospital care

20-60% - Major injury, early hospital care

50%+ - Low salvage, expectant treatment

2. Triage modifiers

a. Inhalation injury

b. Associated injuries

c. Burns of hands, feet, face, and perineum

APPENDIX B

ANIMAL MODELS FOR VESICANT-INDUCED SKIN INJURY

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I. Introduction

My colleagues and I at the Letterman Army Institute of Research (LAIR) have been involved in the development and utilization of animal models for vesicant research since 1981. The following description reflects some of our laboratory experience as well as a review of the literature.

Given the toxicity of many of the agents of chemical warfare that we are interested in studying, it is unreasonable to expect any significant amount of experimentation with humans, although some of the compounds, such as nitrogen and sulfur mustards, are still being used today for the treatment of psoriasis and T cell lymphomas of the skin. Historically, animal models have been our best source of information on toxicity and, to a more limited extent, pathophysiology. In vitro systems are necessary, and certainly desirable, adjuncts to vesicant research, but animal models will be required to investigate most aspects of systemic toxicity and therapeutic efficacy. However, animal models are not without problems.

Disadvantages of Animal Models

1. There are no good animal models for clinically obvious blistering. Very few animals naturally produce vesicles, and those blisters are much smaller than those generated in humans by blister agents. Inability of animal skin to form macroscopic vesicles cannot be explained solely on the basis of skin thickness or appendageal structures. Microvasculature and the intricacies of the inflammatory response may account for the difference in response of animal and human skin. Note that human skin grafted to animals does not vesicate normally.

2. Variability in response is a problem in animal systems. Some animals, such as the rabbit, are reported to be extremely variable in their response to sulfur mustard, whereas other animals may not express certain toxicities at all. The LD_{50}s of animal species vary widely, and some organ system toxicity appears to be species-specific. For example,

the dog exhibits much greater gastrointestinal toxicity and an unusual thrombocytopenic response to mustard. Interpretation of older data is often difficult because of the lack of homogeneity of test animals and failure to sufficiently characterize the experimental hosts. Data must be examined very carefully.

3. Extrapolation of data to humans will always be a problem, and will require a careful matching of specific responses. For example, study of hypersensitivity to mustard should be conducted with an animal such as the guinea pig because many other animals are difficult to sensitize. A particularly difficult problem in the interpretation of therapeutic regimens is the reservoir of free mustard found in some animal skin but not in human skin.

4. Expense and logistics can influence research choices. Even if the horse were the most sensitive and appropriate animal model for skin injury, it would be more difficult to work with than the rat.

5. The animal rights issue is an increasingly serious problem for us in the San Francisco Bay area, despite our strict adherence to USDA guidelines and evaluation of all protocols by an Animal Use Committee.

II. Choosing an Animal Model

The choice of animal model for cutaneous injury depends on a number of factors, the most important of which are the particular biological response one is looking for and the availability of the animals. When searching for an animal model for skin injury, one must keep in mind the tremendous variability in structure and function of the human integument. The whole skin thickness on an individual can vary from 2 mm to nearly 10 mm. There are areas such as the scalp, which is packed with hair follicles, sebaceous glands, and eccrine glands, and there are other areas such as the lip, which is practically devoid of skin appendages. In looking for a suitable animal model for skin injury, we have been impressed with the pig as an experimental host. There are several lines of evidence that would suggest that the pig is a relevant model.

Examination of the relative thickness of skin from several different species, including humans, indicates that the pig has an integument which approximates the thickness of the human. In terms of the density and size of hair follicles, the skin of the pig is more similar to human skin than are those of other species.

Data on skin permeability reveal that the pig may also be a more representative host than other species for studies of human skin penetration. Dr. Reifenrath and his group at LAIR have worked extensively with the porcine skin in vitro model and have demonstrated the suitability of using pig skin as a substitute for human skin in permeability experiments.

Another line of evidence for the value of the pig in vesicant research is the recent results we obtained on the histopathologic changes in pig skin caused by butyl mustard. In hematoxylin-eosin (H&E) stained sections of pig skin 24 hours after exposure to liquid neat butyl mustard (100 ug/cm^2), foci of necrotic basal cells and the beginning of microvesicle formation can be seen. At 48 hours after exposure of pig skin to liquid neat butyl mustard, there is extensive necrosis of basal cells and subsequent vesicle formation. The split occurs at the dermal-epidermal junction, just as described for sulfur mustard injury to grafted human skin.

III. Grafted Human Skin/Nude Mouse Model

Despite the apparent similarities of porcine and human skin, there are some important differences, such as the reservoir of free mustard in the pig skin, that make it less than an ideal model. In an effort to circumvent that problem we have adapted the athymic nude mouse/human skin graft model for some of our vesicant work. The following is a brief description of the model and how we have utilized it at LAIR.

Initially described in 1962, the nude mouse mutant was not known to be athymic until 1968; at that time a great deal of interest was generated in the animal. The immunological abnormalities are severe and relate primarily, although not exclusively, to the cellular immune response. Consequently, the animals will accept xenografts of skin and other tissues from a wide variety of donor species, including humans. Both full- and and split-thickness human skin have been successfully grafted to the mouse, but most of the studies in the literature have been with split-thickness skin.

Four types of skin are used for grafting at LAIR: abdominal, breast, facial, and eyelid from plastic surgery patients. Cadaver skin and postcircumcision foreskin have been used to a limited extent. The large majority of grafts are split-thickness from breast and abdomen. Graft survival is very dependent on thickness, so only the relatively thin eyelid and foreskin are used for full-thickness grafts. Only the full thickness grafts have intact and viable skin appendages such as sweat glands and hair follicles.

To prepare the skin for grafting, the subcutaneous fatty tissue is removed by trimming with a pair of scissors, and then the skin is cut into strips for dermatoming. The skin is then dermatomed to a thickness of approximately 0.6 mm, and circles of skin approximately 13 mm in diameter are cut out for the split-thickness graft.

After general anesthesia with chloral hydrate injected intraperitoneally, the recipient site on the nude mouse is prepared by scissor excision of full-thickness skin from one or both flanks of the animal. This circular defect will exactly accommodate the donor graft, which is then placed into the site and held in place with a piece of sterile Op-Site tape. The tape is removed 5 to 10 days later. In 5 to 6 weeks the grafted mice are ready for experimentation.

Our grafting success varies with the type of skin grafted, its thickness, and the particular group of mice we are working with at the time. Our percent "take" is approximately 70%, and the grafts often survive for the life of the animal, which is 6 months to a year.

At LAIR we have used the grafted human skin/nude mouse model to study the relative permeability of human, mouse, and pig skin. We have also looked at the metabolism of butyl mustard by human skin and we have evaluated the response of grafted human skin to various vesicant analogs. In a soon-to-be-published report, Drs. McGowan and Van Ravaansway from our laboratory describe the light and electron microscopic findings of arsenical damage to human skin grafts. Interestingly, they found that lewisite analogs were capable of producing a subepidermal microvesicle similar to that seen with sulfur mustard. Incidentally, Dr. Papirmeister has also used our grafted animals for his landmark studies on sulfur mustard-induced injury to human skin.

There are other athymic animals which may be valuable in the study of vesicants. One such model is the nude rat, which has many of the same attributes as the nude mouse, with the additional advantages of being larger and hardier. Of particular interest to us at LAIR is the nude rat/human skin flap model developed by Dr. Gerry Krueger and his colleagues at the University of Utah.

This somewhat complicated animal model consists of an athymic nude rat with human skin grafted onto the ventral aspect of the animal. This grafted area and the blood vessels associated with it are then freed and made into a pedicle flap. The pedicle is actually a sandwich of skin, with host skin on one side and grafted human skin on the other. The venous drainage from this flap can be directly accessed for

measurement of skin metabolites and percutaneous penetration of compounds applied to the skin. We have been working with Dr. Krueger and his coworkers and hope to utilize this model for studies on the percutaneous absorption and fate of topically applied chemical warfare agents. (Editor's comment: Nude rats are genetically immunocompetent. To prevent rejection of xenografts, their immune responses are suppressed by the chronic administration of cyclosporine.)

IV. Noninvasive Measures of Skin Injury.

The clinical evaluation and quantitation of cutaneous injury are still major obstacles to the study of vesicant toxicity, regardless of the animal model used. This problem is currently being addressed at our institution by the use of noninvasive measures of skin function. Infrared thermography, laser doppler velocimetry, and reflectance spectrophotometry have been undergoing evaluation in our laboratory. We think that we will be able to improve on the classical Draize testing of skin irritation. We also have most of the equipment necessary for photopulse plethysmography and hope to begin using that too. There are a number of other techniques for measuring skin functions, such as transcutaneous oxygen and carbon dioxide flux, which may be useful in quantitating the effects of vesicants on the skin.

V. Summary and Conclusions

In this brief review I have touched on the major advantages and disadvantages of the important animal models for vesicant-induced skin injury. It is clear that we are far from the ideal model but, by careful selection of specific animals for selected physiological responses, we can gain a great amount of valid data on vesicant toxicity. We still do not have a good model for blistering, but the pig does appear to have a number of advantages over other experimental hosts. The grafted human skin/nude rodent models offer an unprecedented opportunity to study the metabolism and percutaneous penetration of highly toxic compounds in human skin. Last, there is a definite need for better quantitative measures of skin injury, and I urge the exploitation of noninvasive techniques.

APPENDIX C

VESICANT RESEARCH
LETTERMAN ARMY INSTITUTE OF RESEARCH

MAJ Dale R. Westrom, MC

I. Studies on sulfur mustard analog (n-butyl-2-chloroethyl sulfide)

A. Physical and analytical chemistry

1. Development of spectrophotometric and gas chromatographic/mass spectroscopic assays for butyl mustard

2. Study of mustard and its decomposition products in aqueous media using thin layer chromatography

3. Determination of volatility of n-butyl mustard

4. Preliminary studies with mixed organic solvent/water systems using nuclear magnetic resonance (NMR) techniques

5. Application of NMR techniques to determine the kinetics of mustard hydrolysis in detergents

B. Cutaneous metabolism of mustard compounds

1. Evaluation of the effects of butyl mustard on glucose and ornithine metabolism using pig skin and grafted human skin

2. Interaction of skin proteins with butyl mustard

3. Study of skin metabolism of radiolabeled butyl mustard using proportional flow counter with gas-liquid chromatography to measure compounds and metabolites in the skin

4. Determination of percutaneous penetration and cutaneous metabolism using the nude rat/human skin flap model

5. Study of the role of membrane-bound monooxygenases in the sulfoxidation of thioethers

C. Skin decontamination and protection

1. Development of animal models for evaluating the efficacy of decontamination and protective materials

2. Evaluation of decontamination efficacy of selected commercially available skin creams

3. Evaluation of peroxides as skin protectants

4. Development of automated skin permeability model for evaluation of skin protectants and decontaminants

D. Therapy of mustard injury

1. Evaluation of laboratory models (nude mouse, rat, rabbit, pig, grafted human skin) for skin injury

2. Development of noninvasive quantitative measures of skin irritation

3. Light microscopic, electron microscopic, and immunopathologic studies of butyl mustard skin injury

4. Study of the role of plasma proteins in the healing of butyl mustard skin injury

5. Development of topical, intralesional, and systemic therapies for cutaneous and generalized mustard injury using in vitro and in vivo models

II. Studies on lewisite analogs

A. Accomplishments

1. Identified the blood component responsible for systemic dissemination of vesicant arsenicals

2. Determined the biochemical explanation for species differences in systemic transport of vesicant arsenicals

3. Determined the three-dimensional structure of the product of the reaction of phenyldichloroarsine (PDA) with reduced glutathione (GSH) (primary biological target) and the three-dimensional structure of the PDA/British anti-lewisite (BAL) adduct

4. Developed NMR as a tool to study interaction of PDA with intact erythrocytes

5. Developed a gas chromatographic assay for urinary 2,3-dimercaptosuccinic acid (DMSA)

6. Studied the light and electron microscopic changes produced by lewisite analogs on human skin grafts

7. Determined that cultured cells can be rescued with BAL up to 3 hours after challenge with lethal doses of PDA

8. Demonstrated that DNA, RNA, and protein synthesis are all depressed when cultured cells are challenged with PDA

B. Plans (protocols in review)

1. Establish a method to determine binding constants between organic arsenicals and sulfhydryl compounds (potentiometric and calorimetric techniques)

2. Determine the binding constants for PDA with GSH, lipoic acid, BAL, and candidate BAL replacements

3. Compare the binding constants with efficacy in cultured cell systems

4. Extend NMR studies to additional arsenicals and antidotes to obtain information necessary for molecular design of most effective antidote

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